NOV 25 1924

THE JOURNAL OF GENERAL PHYSIOLOGY

Founded by Jacques Loeb

EDITORS

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VOLUME VII, No. 2 NOVEMBER 20, 1924

PUBLISHED BIMONTHLY

AT MOUNT ROYAL AND GUILFORD AVENUES, BALTIMORE, MD.

BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

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Entered as second-class matter November 23, 1918, at the Post Office at Baltimore, Md., under the Act of March 3, 1879.

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TEMPERATURE AND FORWARD MOVEMENT OF PARAMECIUM.*

By OTTO GLASER.

(From the Biological Laboratory of Amherst College, Amherst.)

(Accepted for publication, September 28, 1924.)

I.

Paramecium is extremely sensitive to slight changes in its environment and in consequence has been used, since the days of Spallanzani, as a delicate biological indicator. Yet, despite the three thousand or more papers dealing specifically with this organism, quantitative studies of its relations to the environment have been exceedingly rare and very limited in scope. So far as concerns temperature, for example, we find Pütter (1903),[1] in a curious state of confusion, denying that the production of mechanical energy by this animal is in any way proportional to an increase in heat intensity. Since then, Kanitz (1915)[2] has found in Khainsky's (1911)[3] results on *Paramecium caudatum*, support for his own earlier and even more irregular experiments with the contractile vacuoles of other Infusoria, while Woodruff and Baitsell (1911–12) determined $Q_{10} = 2.84$ or 2.70, for the fission rate over an interval of only 4°. With respect to temperature, certainly, the advantages of sensitivity in Paramecium remain largely unexplored.

The analysis here proposed deals with the effect of temperature on forward motion. This is perhaps the most common act which the animal performs and is, therefore, at all times available for experiment. The purpose of the study does not include an attempt to illuminate the mechanical arrangements that propel the organism;

* Owing to Dr. Glaser's absence in Europe, proof of this paper has been read by the Editors.

[1] Pütter (1903), p. 88.

[2] Kanitz (1915), p. 62.

[3] Khainsky (1911), p. 32.

on the other hand it does involve the employment of temperature effects to detect the basic chemical reactions that supply the energy required for an execution of the ciliary beat.

The movements with which we are concerned result from a rotation dependent on the oblique direction of the ciliary stroke. These cilia are the only machinery of propulsion and a change in activity on their part means a corresponding change in the rate of progress. Under ordinary conditions, therefore, the speed with which the animal spins on its major axis and bores its way through the water may be considered proportional to the rate at which the cilia move. Since, however, we are interested merely in the forward resultant, only the rate of progress of the entire animal was measured; this was done by observing the time required by the organisms to travel a given distance at different temperatures.

II.

In order to minimize the effect of disturbing influences only short distances were considered. Moreover, the organisms were confined in a capillary channel—the readings being made in a locality where the diameter remained constant at 1.5 mm. over a distance of at least 1 cm. The full sized ends of this tube, 10 cm. apart, were bent up at right angles and were clamped in the metal holder provided with two thermometers held parallel with the horizontal runway. If all the adjustments are correct and there are no interfering factors outside, the rate at which the animals move forward in the capillary is entirely consistent and not influenced by direction.

For each set of experiments such a tube was loaded with culture medium—an aerated, filtered extract from 20 gm. of sterile timothy hay which had been boiled for 30 minutes in 500 cc. of water distilled from glass, and to which, after correcting for evaporation, there was added 50 cc. of a Ringer's solution containing, per liter, 6.5 gm. NaCl, 0.14 gm. HCl, and 0.12 gm. $CaCl_2$. The *Paramecia*—all from a single pure line—were then introduced together with enough medium to raise the level in the uprights to a fixed mark. Finally the entire system, with its two concordant thermometers pointing in opposite directions, was submerged to the level of the liquid within the speed-trap, in a water bath provided with a stirrer and controlled by means of a micro burner and, on occasion, by ice.

With equilibrium established after a few moments, the readings were taken with the aid of a black staircase ocular micrometer as the organisms passed through the field of a binocular microscope. For each *Paramecium* the time consumed in covering a certain scale distance was determined by means of a stop-watch. The records of all the animals at a given temperature were then reduced to a common basis, namely the time in seconds required to cover one unit of the scale at a temperature constant within 0.1°C. as given by the average reading of the two thermometers. With a horizontal magnification of × 20, the value of one scale unit in absolute terms was very nearly 500 microns. Every record is the average of not less than six such performances, in each of which the average distance moved by the organisms was six scale units.[4] Perhaps a higher degree of accuracy could have been achieved if the records at all temperatures had been made with a single individual. Certainly the original itemized lists contain variations. At times one finds a "racer" or an individual unusually slow. The effects of deviations of this sort are, however, swamped completely in averaging a large number of observations. This was definitely the outcome of a preliminary statistical analysis yielding the modal time at several temperatures. When it became apparent that such modal points at most differ only in the second decimal from the crude averages, there seemed to be no reason for not employing the latter immediately as data. These are given in Table I and graphically in Fig. 1. They demonstrate not only the ineptitude of Pütter's (1903) remark but also that the process under consideration, like other biological actions, varies as an exponential function of the temperature.

III.

The analysis of this material assumes that forward motion depends on energy derived from a series of chemical reactions, and that to cover a given distance always requires the same amount

[4] The number depended on the length of time during which the temperature remained constant. Many more observations were made at 30° because the first results at this temperature were quite discordant. The difficulty was traced to two sources—a contaminated pipette and a slight difference in the composition of the medium.

of energy. A series of underlying reactions of this sort necessarily has a definite sequence, and the most elementary consideration suffices to show that the rate at which a system of linked processes

TABLE I.

Average Time Required by Paramecium to Swim a Unit Distance at Different Temperatures.

Temperature.	No. of observations.	Time.		
		Observed, average.	Calculated. $\mu = 16{,}000$	Calculated. $\mu = 8{,}000$
°C.		*sec.*	*sec.*	*sec.*
6.6	7	2.64	2.65	
7.0	7	2.58	2.60	
9.9	6	1.98	1.98	
10.7	18	1.89	1.81	
10.8	10	1.80	1.80	
14.8	10	1.22	1.22	1.19
15.0	10	1.17	1.18	1.18
16.0	26	1.10	1.08	1.12
17.5	50	1.04	0.94	1.04
18.0	10	0.91	0.90	1.01
18.5	24	0.89	0.86	0.99
18.7	10	1.09	0.84	0.98
19.0	40	0.95		0.97
19.4	20	0.91		0.95
21.2	10	0.81		0.87
21.5	15	0.71		0.85
22.0	36	0.83		0.83
23.0	32	0.80		0.80
25.0	10	0.74		0.73
25.4	11	0.73		0.72
25.5	15	0.77		0.72
27.0	14	0.65		0.67
27.3	12	0.63		0.65
29.0	40	0.69		0.62
30.0	100	0.56		0.59
39.0	30	0.43		0.41
40.0	7	0.40		0.39

can proceed as a unit depends absolutely on the component with the slowest rate. In its relations to temperature, therefore, forward translation should reflect the corresponding properties characteristic of the controlling reaction. Accordingly, the experimental

points should be calculable by means of the Arrhenius[5] (1915) formula of 1889 which describes the changes in the rate of acceleration of a chemical process at different temperatures and which has been employed with such success by Hecht (1919, 1918–19) in his studies on the influence of temperature on photic sensitivity in *Mya*.

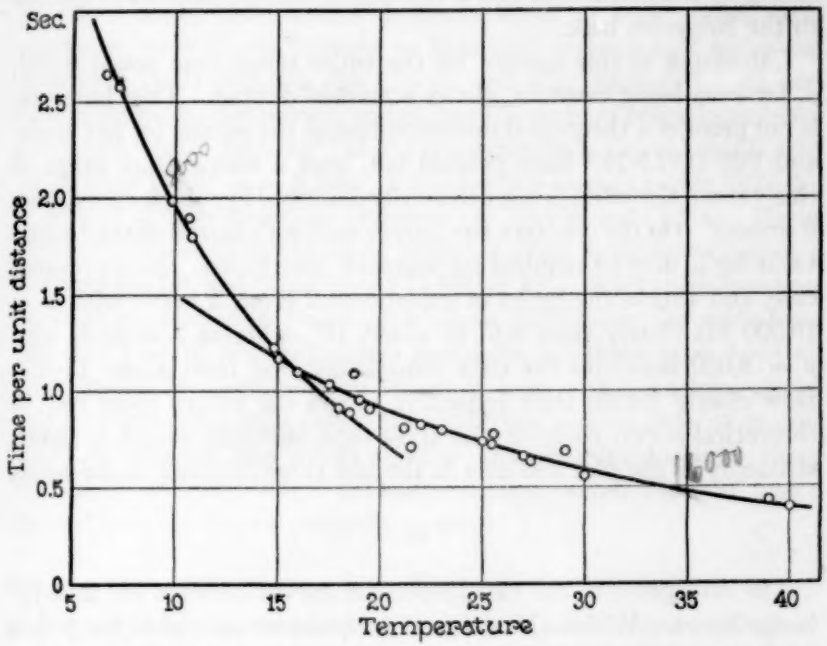

FIG. 1. The Arrhenius equation applied to the relation between temperature and time in the forward translation of *Paramecium*. For the left-hand curve, $\mu = 16{,}000$; for the right-hand, $\mu = 8{,}000$.

In the Arrhenius expression, the ratio between the velocity constants K_1 and K_o of a chemical reaction taking place first at one, then at another temperature is related logarithmically on the Naperian base e to the gas constant, the absolute temperatures, and to a constant μ which characterizes a particular reaction. As formulated,

$$\frac{K_1}{K_o} = e^{\frac{\mu}{2}\left(\frac{T_1 - T_o}{T_1 T_o}\right)} \qquad (1)$$

[5] Arrhenius (1915), p. 49.

From the values in Table I velocity constants cannot be calculated. However, for K_1 and K_2 respectively, we can substitute the reciprocals of the times, $\frac{1}{t_1}$ and $\frac{1}{t_2}$, required to perform the same amount of work. The temperature characteristic, μ, can then be easily determined by transformation and a convenient change from the Naperian to the Briggsian base.

Calculated in this manner for the entire range from about 6–40°, μ, far from being constant, shows a marked decline. This, however, is not proof of a theoretical misconception at the outset, for as Crozier and Pilz (1923–24)[6] have pointed out, over a temperature range of this extent the effect is not necessarily described by a single curve, even if smooth. On the contrary two curves, each with its own characteristic value for μ, may be required for adequate description. In the present case, and within the limits of experimental error, a curve with μ = 16,000 fits closely from 6.6° to about 16°, whereas a second, with μ = 8,000 simulates the data remarkably well from about 15–40°. How closely within their respective ranges the values given by the theoretical curves coincide with those experimentally found, is shown strikingly in the plot and also in the last three columns of the table.

IV.

The divergence of the two theoretical curves between 15° and 19° is significant. Within this range of temperature several of the points might be attached perhaps equally well to either curve; nevertheless, in this region originates a change of direction which above 20° is unmistakable. What does this mean? On the principle of a controlling rate which we have assumed as the basis for our analysis, the problem finds a simple solution. The speed of the entire system of underlying reactions is increased by a rise in temperature, yet it does not follow that all components of the system have the same acceleration. A specific reaction—the slowest one prior to a certain change in temperature—may be replaced by another which even if originally faster, nevertheless with rising temperature drops into the last place.

[6] Crozier and Pilz (1923–24), p. 720.

The idea involved here is not new. As Cohen-Stuart (1912)[7] found it necessary to emphasize, van't Hoff himself expressly stated that in chemical systems Q_{10} remains constant only at certain temperatures. However, the existence and possible significance of a definite break in biological data, particularly at points where the processes under consideration begin to occur at supernormal temperatures, were clearly brought to light only recently by Crozier and Federighi (1923–24) (see also Crozier and Pilz, 1923–24[9]). In certain arthropods, the process underlying the state of reflex immobility (so-called "death-feigning") at low temperatures has a μ value of 24,000, while from 16–30°, μ = 9,200. For other processes (Crozier, 1924–25, *a*, *b*, and *c*) corresponding extremes are 16,400 and 11,200. Hecht (1918–19) reported for the latent period of *Mya*, in the lower ranges, a value of 19,000, and slightly over 11,000 at 31°. With these instances forward translation in *Paramecium* aligns itself easily, for aside from the fact that in *Mya* μ declines gradually, all these cases have all these points in common: high values of μ go with low temperatures and *vice versa;* the high values fall within one general order of magnitude, and the low ones within another; and when breaks occur, these are found abruptly where the temperatures exceed those at which the animals or tissues normally operate.

V.

For our analysis the physical meaning of μ has important consequences. Historically this constant traces back to 1872 when Guldberg (Arrhenius (1912)[8]) found the rate of change in the logarithm of the dissociation pressure directly proportional to the heat of dissociation, and inversely, to the gas constant and to the square of the absolute temperature. The expression of this was applied by van't Hoff in 1885 to chemical velocities, and in 1889 appeared the Arrhenius simplification. The descent of μ in the theoretical line, then, is perfectly clear.[9] In its latest guise it bears the original rela-

[7] Cohen-Stuart (1912), p. 1162.

[8] Arrhenius (1912), p. 83.

[9] In his reference to the empirical equation of Arrhenius, Rice (1923, p. 2808) does not distinguish sufficiently between a formula which is truly empirical and one whose use, even if discovered by arbitrary methods, nevertheless depends on constants thoroughly grounded in physical theory.

tions to the absolute temperature and the gas constant. As used by Arrhenius, therefore, μ fundamentally stands for, and in the most favorable data is proportional to, and perhaps identical with, a heat of dissociation or reaction.

Quite recently, F. O. Rice (1923) has brought forward considerations which if fully substantiated must greatly increase the utility of μ. Arrhenius explained the effect of temperature on the hydrolysis of cane-sugar by HCl in watery solution by postulating that the sugar was present in two forms, the active and the inactive, in mass action equilibrium, and that at equilibrium the concentration of the active form was very low. Rice assumes that in acid catalysis the H ion rather than the sugar exists in two forms, the hydrated ion and the unhydrated, and that the latter is the active form and is present in low concentration because of the great affinity between the H ion and water. An exactly comparable assumption is applied also to hydroxyl ion catalysis. In this way it appears possible to explain why μ has practically identical values for so many reactions superficially different. Far from being a meaningless accident, the fact becomes rationally clear if we follow Rice's suggestion and focus our attention on the chemical mechanism by which transformations are brought about rather than as heretofore on the primary substances transformed (Crozier, 1924–25, *a*, *c*). If this point of view is correct, chemical reactions must group themselves into a relatively small number of classes, and each class must possess a characteristic μ, because in each class of reactions the mechanism involved is the same.

In his analysis of the data on the mammalian auricle, echinoderm eggs, the breathing rhythm of insects, the reduction of hemoglobin, and respiration in nervous tissue and in bacterial processes where the velocities measured are probably those of oxidation, or at least, are functions of respiration, Crozier (1924–25, *a* and *b*; Crozier and Federighi, 1923–24) has found consistently values in the neighborhood of 16,000. The quite independent discovery of $\mu = 16{,}000$ for forward translation in *Paramecium* suggests, therefore, an oxidation as the controlling reaction for the lower ranges.

In the present state of our knowledge an attempt at the class identification of the reaction whose rate of acceleration is measured by $\mu = 8{,}000$ is perhaps even more hazardous. In order of magni-

tude, this value might not fall outside the class of hydrolyses. It is true that Rice (1923) finds 10,950 from Reicher's data on the hydrolysis of ethyl acetate by sodium hydroxide and from Wilsdon's studies on the hydration rates of acetic, propionic, and camphoric anhydrides, an average of $\mu = 11{,}100$. On the other hand, in a list of μ values for a variety of processes, Arrhenius[10] includes 7,540 for the saponification of cottonseed oil by the lipase of castor beans, while for the tryptic cleavage of casein, $\mu = 7{,}400$. Until we have much more definite information in this field, commitments regarding specific reactions are hardly warranted.

VI.

In conclusion we may ask whether it is possible to conceive a chemical reaction system capable of accounting for the distribution of the data and for the apparent transfer of velocity control from one process to another in different temperature ranges.

The observations themselves and their analysis do not limit us particularly to any specific mechanism. The simplest conception applicable to the controlling reactions is a catenary series $O \rightarrow A \rightarrow E$ in which an original source of supply O is changed into the available form A, whose destruction to the end-products E, yields the energy resulting in translation. If $O \rightarrow A$ has a μ value of 8,000 while for $A \rightarrow E$, $\mu = 16{,}000$, the depletion of A at higher temperatures would proceed at a faster rate than its replenishment, and hence the increase in the rate of the process as a whole would become less as time went on.

As an explanation, this scheme cannot be supported by the sort of evidence that would rule out every other possibility. We may in fact be dealing, at the higher temperatures, with the increasing effect of some process that interferes with the flow of $A \rightarrow E$ by diverting a fraction of A. Indeed, the data apparently will bear an interpretation similar to the one which led Hecht (1918–19) to postulate a heat inactivation in connection with the photic response of *Mya*. Such a mechanism, however, is not so simple, and the data for *Paramecium* do not enable us to decide between these two possibilities.

[10] Arrhenius (1915), p. 54.

While the less complicated idea accounts for the facts and hence is preferable as a first approximation, the mere dealing with velocity controls only, lends a deceptive simplicity to the suggested mechanism. As a matter of fact, if we consider A in the catenary series a reservoir in the main supplied from O, and consider E as partly oxidized and partly reversible to O, Meyerhof's conception of carbohydrate metabolism in muscle becomes quite applicable (Shaffer, 1923). *Paramecium* undoubtedly also oxidizes carbohydrates, and unless the metabolism is radically different, the process runs in a cycle and is composed of two main steps. In muscle, according to Meyerhof, glycogen is hydrolyzed to glucose and lactic acid during the anoxidative phase, whereas the oxidative results in the oxidation of both these products as well as in the reconversion of three-quarters of the lactic acid to glycogen. If we attach the lower μ value (8,000) to the synthesis of glycogen from lactic acid—an assignment reasonable in view of the large quantities of water produced during this phase—Meyerhof's conception should give a fairly well substantiated reaction system capable of the velocity controls demanded for the present case in *Paramecium*. Moreover, Meyerhof's circular scheme accounts well not only for the transfer of control but allows for the fact that the data can be interpreted as involving at the higher ranges a deficit of A. If we assume for normal temperatures a quantitative adjustment such that the prevailing rate $O \rightarrow A$ keeps the concentration of A constant at the prevailing rate of oxidation and of the reaction $A \rightarrow E$, and then assign $\mu = 16{,}000$ to the oxidative phase, temperatures lower than normal would result in no change of control, but possibly in an accumulation of A. At higher ranges on the contrary, owing to the depletion of A, the rate of the system as a whole would come to depend more and more on the synthetic transformation of E to O, the forerunner of A, and thus, concretely, if *Paramecium* were a muscle, on the conversion of the lactic acid back to glycogen. Obviously such details must await further investigation. The available data merely bring the analysis of forward translation of *Paramecium* into the sphere of physical-chemical methods and conceptions.

SUMMARY.

1. The rate of forward movement in *Paramecium* as affected by changes in temperature can be described accurately in terms of the Arrhenius equation.

$$\frac{K_1}{K_o} = e^{\frac{\mu}{2}\left(\frac{T_1 - T_o}{T_1 T_o}\right)} \quad (1)$$

2. For the range from 6–15°, μ = 16,000; from 16–40°, μ = 8,000. These values fall within the limits characteristic for chemical processes.

3. On the principle of velocity control by the slowest rate, it is assumed that in *Paramecium* at temperatures above normal, control passes from one underlying reaction to another.

4. The views expressed by Rice, the recent results of Crozier, and certain μ values given by Arrhenius all suggest that μ = 16,000 may represent an oxidation, and μ = 8,000 either a modified oxidation or an hydrolysis.

5. For the system of controls, the catenary series $O \rightarrow A \rightarrow E$ with the lower μ value attached to the precursor reaction is adequate. We may also assume a cyclical system analogous to Meyerhof's conception of carbohydrate metabolism in muscle. In this case it is necessary to assign μ = 16,000 to the oxidation of A and E and μ = 8,000 to the synthesis $E \rightarrow O$. This model also accounts for the fact that the data might be interpreted as involving, apparently, a depletion of A at the higher temperature.

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ON BIOLOGICAL OXIDATIONS AS FUNCTION OF TEMPERATURE.

By W. J. CROZIER.*

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(Accepted for publication, September 28, 1924.)

I.

The use of the critical thermal increment (μ of Arrhenius, 1889) for the characterization of biological processes whose velocities are a function of temperature has been discussed in several preceding papers (Crozier, 1924–25; Crozier and Federighi, 1924–25, *a* and *b*). It was pointed out (Crozier, 1924–25) that in the case of oxidative phenomena critical increments of the orders 11,500 and 16,700 were repeatedly encountered. As reason for the occurrence of *both* these increments in connection with a single phenomenon (tissue respiration), one on either side of some median temperature (usually found to be near 15°), it was suggested that at least two processes might be concerned in biological oxidations. Thus the virtual velocities of two catenary reactions

$$A \xrightarrow[k_1]{} B \xrightarrow[k_2]{} C$$

with velocity coefficients k_1 and k_2, and having different temperature characteristics, might be so related that at a certain temperature these actual velocities are dynamically identical, while below that temperature $A \rightarrow B$ would be the "slow reaction," and above that temperature $B \rightarrow C$ (or *vice versa*).[1] In discussions of "temperature

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[1] There are a few hints obtainable from reactions *in vitro* to the effect that this situation may be duplicated in simpler two-stage reactions, but such phenomena have been very incompletely investigated. In certain enzymatic processes this change of μ may be shown, as in connection with saccharase (von Euler and Laurin,

coefficients" the rôle of the slowest process of a catenary set as the master process of the whole is sometimes insufficiently appreciated; two simple illustrations of it are given in Figs. 1 and 2.[2] It follows, on this view, that in a catenary or other complex system in which several reactions are involved, the critical increment may be different above and below a certain temperature. It is assumed that the critical increment refers to, and is characteristic of, the formation of active molecules or ions of a catalyst.

This general view-point serves to make it comprehensible, as Rice (1923) has strikingly shown for catalyses by the ions of water, that quite diverse phenomena (provided they have the same catalyst) should yield the same critical thermal increment. The empirical fact is otherwise quite mystifying. It has been known for some time that the same reaction exhibits different temperature coefficients when activated by different catalysts (*cf.* Slator, 1903; Plotnikow, 1907). The temperature characteristic, μ, is independent of the *amount* of a catalyst (*cf.* Lamble and Lewis, 1914, etc.). The constant μ is subject to slight variations with temperature, but these changes, and those due to ion effects (*cf.* Rice, 1923; Rice and Kilpatrick, 1923; Rice and Lemkin, 1923) are of so small an order as to interfere but slightly with the analysis of biological systems. The chemical composition of living matter is so nearly uniform, and operates under the control of such efficient regulatory mechanisms, as to be distinctly favorable for the application of the concept of critical increments. On the other hand, the processes measured are bound to be far from simple. It will be

1920); and there possibly is indication of it in the data of Hudson and Paine (1910) upon the destruction of invertase by HCl. For biological systems Kanitz (1915) has suggested that a different relation to temperature may obtain in different portions of the temperature range; but he failed to make precise systematic use of the idea, since he was mainly concerned to justify the "Q_{10}" rule.

[2] It is not a simple matter to illustrate this rule in detail by examples from general chemistry. Perhaps the nearest approach to a suitable case is found in the stepwise hydrolysis of polyesters. As worked out by Meyer (1909, *a* and *b*) and Yamasaki (1920) the relations between the velocity coefficients for the removal of successive symmetrical ester groups bear, however, an integral relation to one another, and they have the same temperature characteristic (which for the action of acid and of alkali are usually those associated with processes respectively catalyzed by H' and OH^-, or with acid hydrolyses).

pointed out that under different conditions one and the same activity may indeed show quite different critical increments. From the standpoint that such a resultant as the frequency of heart beat, for example, is determined by the action of a catenary chain, this finding is not at all unreasonable.

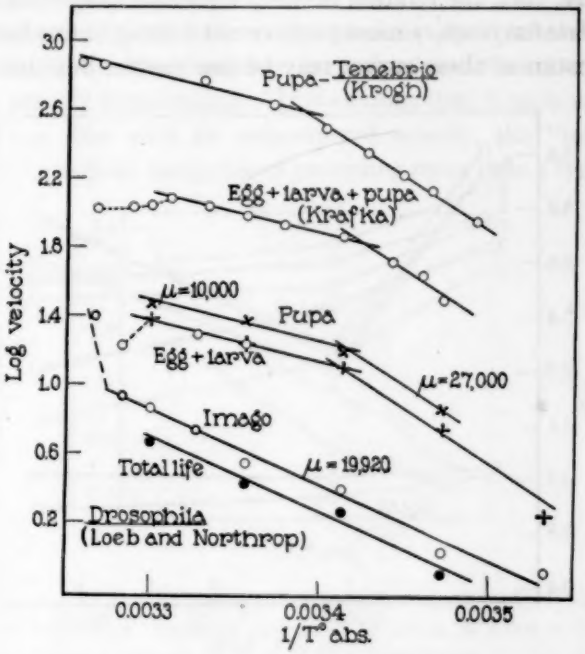

FIG. 1. The four lower curves, derived from the measurements of Loeb and Northrop (1917) with aseptic animals, give the velocity of development and of death for egg + larva, pupa, and imago of *Drosophila*, and for the total life span; Krafka's (1919–20) figures on ordinary culture flies (not aseptic) show quantitative agreement. In the *egg + larva* curve two sets of measurements (different symbols) have been brought together by multiplying the velocities in one set by a factor; this has also been done with the two uppermost curves, so that they might be conveniently included in the same figure. In the case of *Drosophila* temperatures above 30° have a destructive effect. The critical increments for the stages involving growth and differentiation are the same in all cases, and reappear in Krogh's (1914) data on the rate of pupal development in *Tenebrio*. The increment for the reciprocal of imaginal life duration is different from those involved in the control of embryonic and larval stages; *the velocity of the controlling life process is lowest in the case of the imagal stage, and its critical increment determines that of the total life span.*

It would be quite a mistake to suppose that all process varying regularly with temperature should yield to this method of direct analysis. Two important exceptions may be cited. When an end-result is the expression of an equilibrium condition, the outcome of a balance between opposed or consecutive reactions differently influenced by temperature, then the relation between *logarithm of effect* and *reciprocal of absolute temperature* must produce not a straight line but a curve. In some instances these curves may be analyzable, but this need not

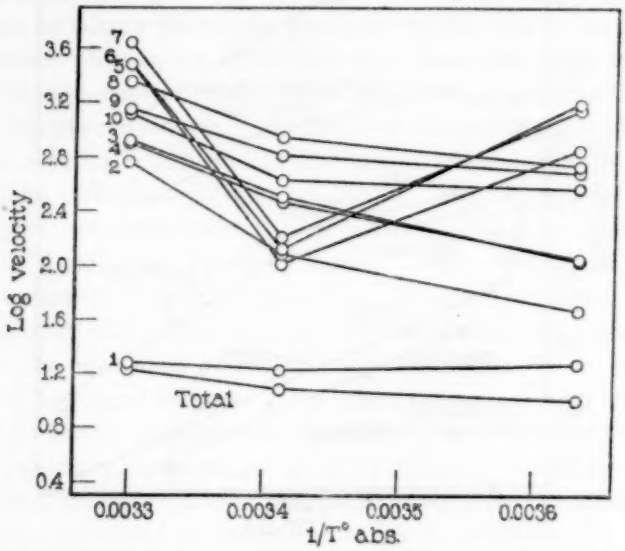

FIG. 2. The velocities of ten successive stages in cell division in onion root tip cells, determined by a statistical method (Laughlin, 1919), and of the total process. Stage 1 has the lowest velocity, and (within the limit of error of the determination) impresses its temperature coefficient upon that of the total velocity for mitosis.

be gone into here. Very pretty illustrations of the effect are provided by measurements of the activation of sea urchin eggs by acid at different temperatures, and by the relation between temperature and number of eye-facets in *Drosophila*.

R. S. Lillie (1917) in two series of experiments measured the "optimum" time for exposure of *Arbacia* ova to acid, at different temperatures, the optimum being defined as that duration of exposure providing the greatest proportion of larvæ. The reciprocal of this

exposure time measures the velocity of the underlying parthenogenetic process, at the point where "activating" and "destructive" actions of the acid are (statistically) in a maximum equilibrium. The data (Fig. 3) obviously fall upon intersecting curves. The intersections occur at temperatures known from other studies to be "critical" temperatures for these eggs.

The numerous quantitative observations upon the relation of temperature to eye-facet number in various genetic races of *Drosophila*[3] are equally illuminating. It is obvious that in such cases, since one does not deal with an unhampered *velocity*, the "temperature coefficients" are quite incapable of providing more than a crude indica-

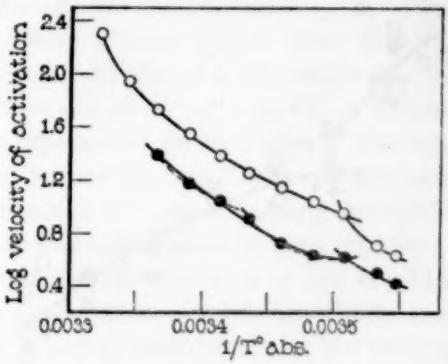

FIG. 3. Two series of experiments, data from R. S. Lillie (1917); log ordinates, reciprocals of "optimum" exposure (minutes) to action of 0.006 N butyric acid in sea water; abscissæ, reciprocals of absolute temperatures.

tion that chemical forces are at work, a conclusion self-evident in the first place and which therefore is not especially instructive. The bar gene acts as an inhibitor of eye-facet development, the amount of eye reduction being greater at higher temperatures. One must therefore deal, not with the *number* of eye-facets, but with the efficiency of the reduction; this may be measured either as percentage reduction, or better by the *reciprocal of the number of facets*. I have carried out this procedure with the available facet counts in several bar and ultra-bar stocks, and in the various F_1 heterozygotes. The figures for the

[3] Seyster (1919), Krafka (1919–20), Hersh, R. K. (1924), and Hersh, A. H. (1924).

various series fall upon parallel curves, so that the multiplication of each of the sets of measurements by a suitable factor brings them all into coincidence (Fig. 4). The points fall upon two intersecting curves, which cross at near 27°C. An analysis of the latitudes of variation shows that on either side of 27° these follow a different course, and confirms the result given in Fig. 4. The fact that temperature influences so slightly the number of facets in the full-eye races (Hersh, R. K., 1924) is further proof, if such were needed, that the facet number is determined by an equilibrium. We may conceive that the

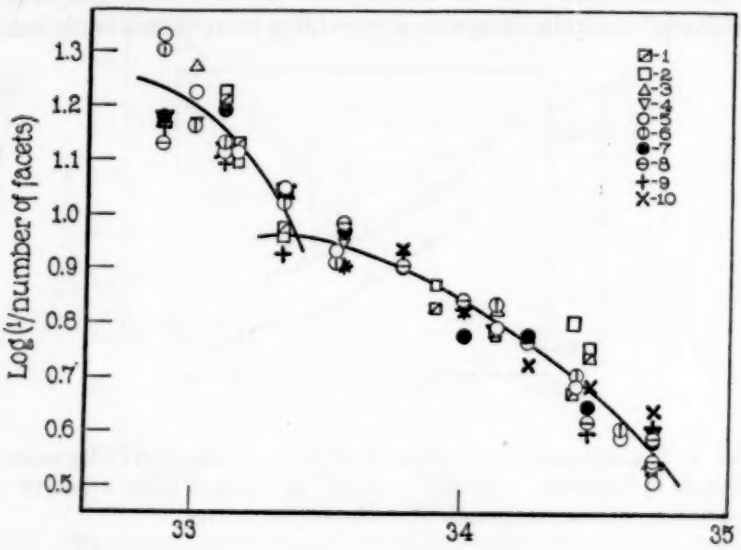

FIG. 4. The logarithm of the reciprocal of the number of eye-facets in mutant races of *Drosophila* containing the bar gene, plotted against reciprocals of absolute temperatures. The different series of measurements have been brought together for comparison by multiplying the determinations in each set by a factor. The different series are:

Nos. 1, 2, Red bar-eye ♀ and ♂ (Seyster, 1919).
" 3, 4, White bar-eye, ♀ and ♂, unselected (Krafka, 1919–20).
" 5, 6, " " ♀ " ♂, low-selected (Krafka, 1919-20)
No. 7, Bar, ♀ (Hersh, 1924).
" 8, Ultra-bar, ♀ (Hersh, 1924).
" 9, F_1 ♀ Bar × Ultra-bar (Hersh, 1924).
" 10, F_1 ♀ Full-eye × Ultra-bar (Hersh, 1924).

number of facets is determined by the amount of a formative catalyst, B, normally existing at a definite concentration which results in a system of the type

$$A \underset{k_1}{\longrightarrow} B \underset{k_2}{\longrightarrow} C,$$

in which k_1 and k_2 have not very different temperature characteristics. But in the bar-eyed race the amount of this "formative substance" is reduced, owing to the alteration of the reaction $A \rightarrow B$. The most convenient assumption would be that the bar mutant gene produces a negative catalyst for the process $A \rightarrow B$; this would automatically reduce the equilibrium concentration of B and at the same time increase the value of μ for the reaction $A \rightarrow B$. Change of temperature would thus have a much greater effect than in the wild stock. (It must also be assumed that A is differently affected, by subsidiary processes, on either side of 27°; *cf.* Fig. 4.) I wish merely to indicate the nature of an explanation for such cases. The relation of ultra-bar to bar (Morgan, Sturtevant, and Bridges, 1924), and the dominance effects on either side of 27°, are apparently susceptible to analysis in this way; but the matter need not be gone into here, beyond stating that the nature of the ultra-bar race as one in which two *bar* genes are present in a single chromosome is beautifully consistent with the observed similar effects of temperature on the two races. It may be pointed out that the type of equilibrium result seen in the case of the sea urchin eggs treated with acid, where *competitive* effects of the acid are involved, yields an instructively different sort of picture from that derived in the *Drosophila* instance, where the *balance* of consecutive processes is thought to be involved; the curves are, in the first case, convex to the temperature axis; in the second case, concave thereto. Where parallel reactions are involved log K will show positive acceleration with increasing temperature; when the equilibrium concentration of an intermediate compound is measured, it may show negative acceleration as the temperature rises.[4]

[4] The use of this equation will only lead to unnecessary confusion unless its physical meaning is carefully kept in mind. Professor E. N. Harvey has called my attention to the fact that in Feldman's treatment of the subject (Biomathematics, 1923, p. 236) there occurs a devastating error whereby the van't Hoff equation for shift of *equilibrium constant* with change in temperature is identified with the

The present paper is intended to bring together, from the standpoint of critical increments, data on several oxidative processes in animals and plants, and to give a tentative explanation for the values of the critical increments found.

II.

It is desirable for an inquiry of this sort to be in possession of data obtained at short intervals of temperature, and to have the measurements of reaction velocity so numerous that the variation in velocity at constant temperature may itself be investigated. Few available sets of measurements satisfy these requirements, and interpretations may thus be, at times, in error. But even so it is remarkable to what extent the temperature characteristics of diverse respiratory phenomena, and of other processes demonstrably dependent upon oxygen tension (and thus governed by respiration) do show empirical agreement. The method of analysis developed in this connection (*cf.* Crozier, 1924–25) is strikingly justified by the manner in which the data supplied by independent experimenters, having themselves no thought of this theory, are reducible to systematic and rational order.

Figs. 5, 6, 7, and 8 show the relation to temperature as obtained from published observations upon CO_2 production and O_2 utilization. The resulting values of the critical thermal increments are summarized in Table I. For comparison, data on fermentation by yeasts (Fig. 9), and on photosynthesis, have been included.

equation for change in *velocity constant*. The Q in the van't Hoff equation, signifying heat of reaction, has nothing directly to do with the quantity μ; one mode of explanation which makes this clear is given by Lewis (1917). This error leads Feldman in a later paragraph (p. 237) into an absurd calculation of the "temperature coefficient" for the hydrolysis of ethyl butyrate on the basis of the heats of combustion of the reactant and resultants; so that not only is heat of reaction confounded with heat of activation, but equilibrium constant is regarded as a velocity coefficient!

In the light of the discussion in the text it might be suggested that in some instances where two intersecting lines are drawn in the figures the data might better be fitted by a curve. If this were so one would not expect exact agreement with cases where the whole temperature range is adequately fitted by a single straight line. It will be noticed, however, that the agreements are quantitative.

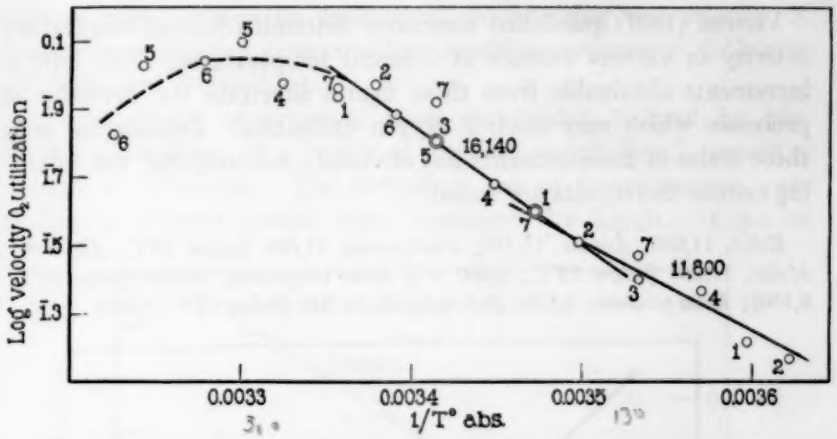

FIG. 5. Oxygen consumption of *Arbacia* eggs (data of Loeb and Wasteneys, 1911). Measurements from seven sets of experiments (1 to 7) have been brought into juxtaposition by the use of a factor for each set. "Critical points" occur at 14°C. and about 25°C. The somewhat fragmentary data provided by Warburg (1908, 1914) for ova of other echinoids are not inconsistent with the assignment of the increment illustrated in this figure for the normal range of temperatures. The lower "critical point" coincides with the lowest temperature for normal development, but there is evidence sufficient to demonstrate that oxidative phenomena do not directly control the rate of cleavage.

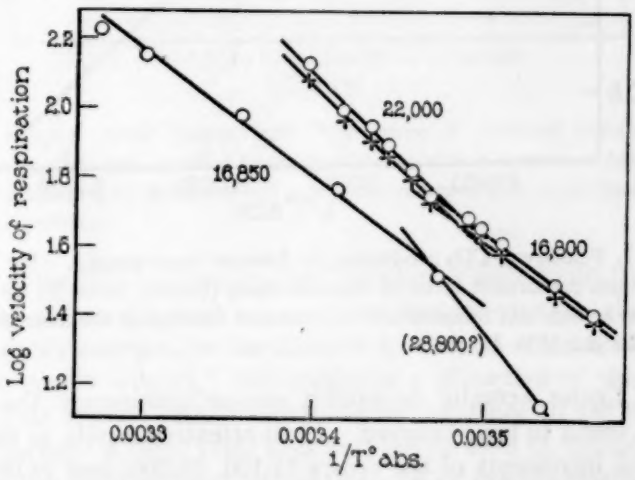

FIG. 6. Upper graphs, O_2 utilization (crosses) and CO_2 production (circles) of starved crayfish (data of Brunow, 1911); lower, O_2 utilization of *Tenebrio* pupæ (Krogh, 1914).

Vernon (1897) published numerous determinations of respiratory activity in various animals at different temperatures. The critical increments obtainable from these figures illustrate the diversity of processes which may control oxygen utilization. Considering only those series of measurements not obviously too irregular, the following critical increments are found:

Helix, 11,080; *Anguis*, 15,350; *Amblystoma*, 11,080 (below 15°C., 8,000 ±); *Molge*, 11,080 (below 15°C., 8,000 ±); *Rana temporaria*, 24,200 (below 15°C., 8,160); *Rana esculenta*, 8,160; *Bufo vulgaris*, 24,200 (below 15°C., 8,160).

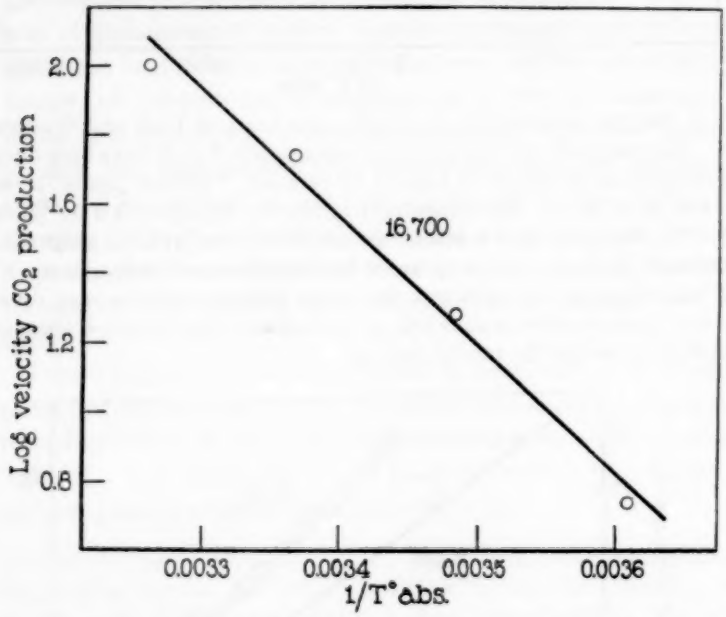

FIG. 7. Velocity of CO_2 production by *Limulus* heart ganglia. The points are means from concordant series of measurements (Garrey, 1920–21) in which the variation at constant temperature is a constant fraction of the mean (*cf.* Crozier and Federighi, 1924–25, *a*).

The figures actually do exhibit greater consistency than Krogh (1916) seems to have believed. I call attention merely to the occurrence of increments of the orders 11,100, 16,000, and 24,000, these having been demonstrated in cases already presented; and to the discovery of 15° as again a "critical temperature." The increment

8,000± may perhaps be understood as reflecting the velocity of central nervous activity concerned in breathing movements (*cf.* Crozier and Federighi, 1924–25, *b*).

Krogh's (1916) observations upon vertebrates, utilized by him for the construction of a "curve of standard metabolism," present a number of difficulties. The individual sets of measurements, pertaining to different species, were "averaged" by Krogh. It can be

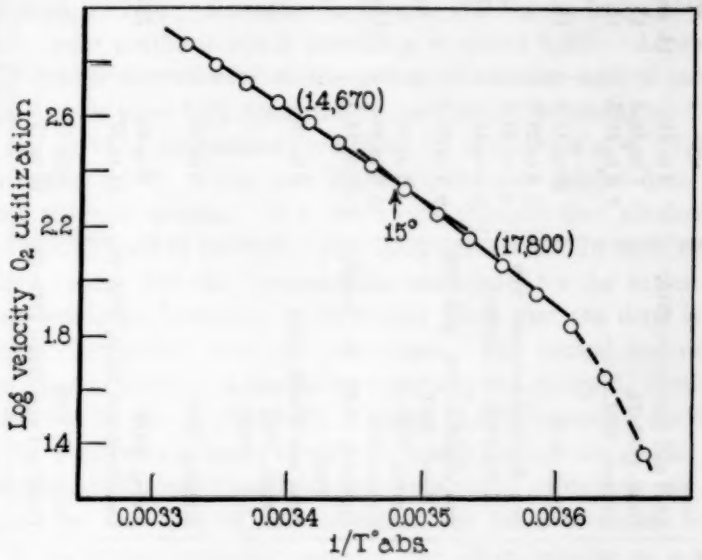

FIG. 8. Krogh's (1916) figures from "the curve of standard metabolism." The determinations were derived from experiments with a variety of forms, the points being taken upon the smoothed curve; the values of μ, in parentheses, have no precise significance.

shown that the "critical temperature" at which a shift occurs from the dominance of one thermal increment to that of another, does not remain exactly constant for the different species; when series of determinations are "averaged," this results in a distortion of the fitted curve. This is most simply illustrated by reference to the case of *Tenebrio* larvæ. Krogh (1916) says that the curve of O_2 consumption of these pupæ as function of temperature differs fundamentally from the "curve of standard metabolism." Fig. 8 demonstrates that the

TABLE I.

Object.	Observer.	Temperature range.	Critical temperature (when evident).	Lower temperatures.	Higher temperatures.
		°C.	°C.		
Arbacia eggs (O_2 utilization).	Loeb and Wasteneys, 1911.	3 to 31	14, and 25	11,800	16,140
Mytilus gill epithelium (O_2 utilization).	Gray, 1923–24.	1 to 34.5	15	16,700	11,590
Crayfish, starving (O_2 utilization and CO_2 production).	Brunow, 1911.	7.6 to 21.5	15.2	22,000	16,800
Tenebrio pupæ (O_2 utilization).	Krogh, 1914.	10 to 32.5	15.5	(28,000)	16,850
Dixippus adults (O_2 consumption and CO_2 production).	von Buddenbrock and von Rohr, 1922–23 (3 series).	2.5 to 20.9		16,200	
		4.5 to 33.5	17	(29,500)	11,500
		9.5 to 31	17±	16,300	11,000
Cosmotriche larvæ, *Antherea* larvæ (O_2 consumption).*	von Buddenbrock and von Rohr* 1922.	9.1 to 29.7		16,000±	
Limulus heart ganglion (CO_2 production).	Garrey, 1920–21.	5 to 34		16,700	
Guinea pig uterus (O_2 utilization).	Evans, 1923.	12.5 to 40	(15±?)	——	11,500
Carp (O_2 utilization).	Lindsted, 1914 (in Krogh, 1916).	1.25 to 25.1		16,500	
Goldfish (O_2 consumption).	Edge and Krogh, 1914.	0 to 28		16,100	
Toad (O_2 utilization).	Krogh, 1914.			16,800	
Winter frog (O_2 utilization).	" 1914.			21,000	
Pisum (CO_2 production).	Kuijper, 1910.	0 to 26		16,200	
Yeast (CO_2 production).	Slator, 1906.	5 to 40	22.5	22,200	12,250
Ulva (CO_2 production).	Osterhout and Haas, 1918–19.	17 to 27		——	12,400
Pisum (CO_2 assimilation).	Matthaei, 1904.	6 to 37	16.0	12,920	10,290
Ulva (CO_2 assimilation).	Osterhout and Haas, 1918–19.	17 to 27		——	10,300

* Determinations upon a number of other insect larvæ and pupæ, plotted by von Buddenbrock and von Rohr (1922) are somewhat erratic, but yield results probably not inconsistent with these.

only difference of any moment has to do with the portion of the curve above the critical temperature (*cf.* with Fig. 6).

Another serious difficulty with these measurements concerns the possible effect of anesthetics and narcotics, used to eliminate muscular movements during the experiments. For toad, and frogs under urethane, Krogh's figures yield μ = 17,000; under curare the critical increment seems unquestionably lower (for frogs, μ = 15,000; for dogs, 13,780). Knauthe (in Krogh, 1916) gives irregular data on carp under urethane which provide μ = about 9,400. According to Hill (1909) the velocity of the action of nicotine and of curare on frog muscle have high temperature coefficients; assuming no "break" in the curve of temperature relations, for the former μ = 17,300; for the latter, 6,500. Veley and Waller (1910) give similar data for the toxic effect of quinine. It is not a new thought that alkaloids may act as catalysts of oxidative and other processes. In such event we might expect that the "temperature coefficient" for the action of the drug would be incapable of furnishing proof that the drug is "combining chemically" with the protoplasm. The critical increment for the effect of nicotine in producing mantle spasm in squid, from figures published by Moore (1918–19) is about 17,800 (assuming no "break" in the temperature range 14–24°, for which no data are given). If an introduced alkaloid should act as a catalyst of oxidations, we should expect to find the critical increment for these processes lowered; or if, as certain evidence suggests, the drug behaves as a *negative* catalyst, the temperature characteristic must become higher.

A point possibly significant involves the high increments found in some experiments with frogs (Vernon, 1897) and in Krogh's data in a winter frog (21,000 to 24,000); also, in the higher part of the temperature range, in the case of Brunow's tests with a starving crayfish (Table I). According to Morgulis (1923) laboratory confinement of *Panulirus* results in the rapid disappearance of glycogen from the blood. It is not difficult to conceive that under these and similar circumstances involving inanition, the limiting conditions for oxidative processes may be profoundly modified. It is tempting to regard these high values of μ as due to the controlling influence of hydrolytic reactions which prepare the substrate of oxidations.

Not very much weight can be put, then, upon the "curve of standard metabolism." The examples of respiratory velocity most suitable for

analysis show unmistakably the presence of controlling reactions with critical increments μ = 11,500 and 16,800; possibly an increment 16,200 is also to be distinguished.

The fermentative production of CO_2 by yeasts (Slator, 1906) gives μ = 12,250 (22.5 – 40°) and 22,200 (5–22.5°); the data are plotted in Fig. 9. It may not be an accident that 12,300 is the critical incre-

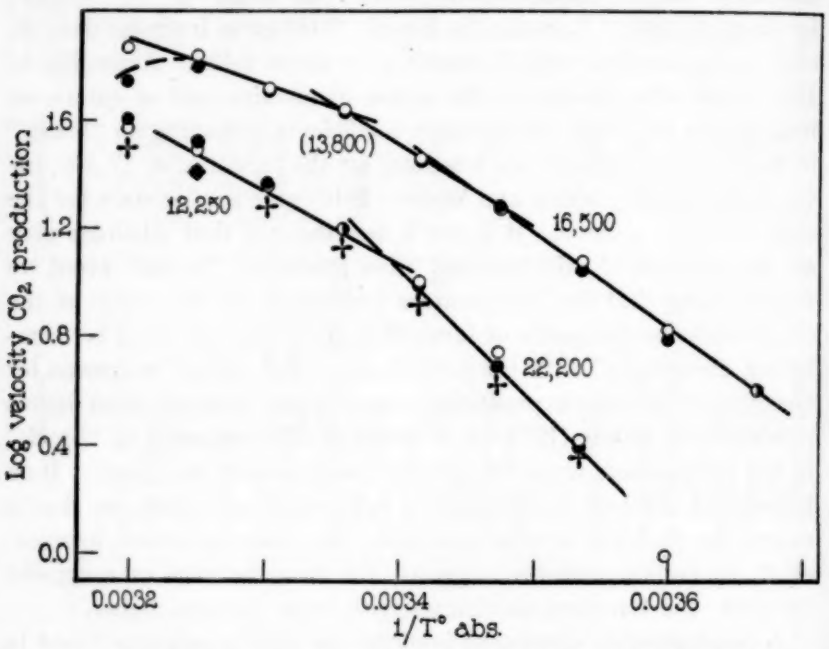

FIG. 9. Upper curve, measurements by Kuijper (1910) of CO_2 production by *Pisum;* white circles, 1st hour, black circles, 2nd hour of exposure to designated temperature.

Lower curve, data by Slator (1903) on velocity of CO_2 production (fermentation) by three types of yeast (different symbols).

ment for the action of certain diastases. This value agrees with that calculated from experiments by Osterhout and Haas (1918–19) upon CO_2 production by *Ulva.*[5] The increments for CO_2 assimilation by plants are definitely different, so far as the available data indicate (Table I).

[5] The increment 12,300 derived for these processes may be suggestive in relation to Palladin's theory (1913) of a preliminary anaerobic stage in plant respiration.

III.

Examination of measurements which have been published upon a number of organic activities shows that they provide critical increments very similar to those given for oxidative phenomena. Since it is possible to show in certain instances that an activity in question is a function of oxygen tension, or in other cases may safely be presumed to involve cell respiration, the agreement of the values of μ is scarcely to be conceived as accidental.[6]

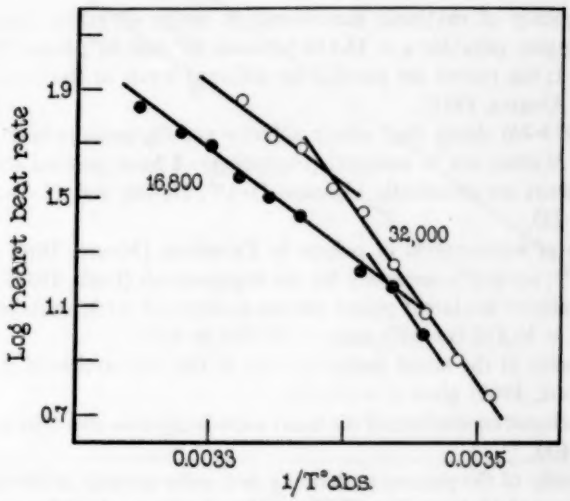

FIG. 10. Martin's (1904) data on heart beat frequency; white circles, heart perfused with Ringer solution; black circles, perfused with Ringer solution *plus* 0.08 per cent KCl. The process having μ = 16,800 is alone materially affected by the KCl.

1. The critical increment for frequency of contraction in the isolated auricle of the rabbit (Clark, 1920–21) is 16,000. Clark and others stress the extreme sensitivity of the auricle to O_2 deficiency.

2. For a good number of instances of heart rhythm μ = 16,800 ±. These I shall consider in a later paper. The spontaneous rhythm of strips from septal and nodal regions of the heart gives μ = 16,410 (Moorhouse, 1912–13). One case of

[6] Arrhenius (1907, 1912) has commented in a general way upon the similarity of the values of μ calculated for very diverse processes, but he offered no explanation.

particular interest is given in a paper by Martin (1904); in his data the value of μ for heart beat frequency is 16,800, above 22°±; Martin shows that if the potassium in the perfusing fluid be increased, the heart frequency is proportionately lowered. The value of μ is the same for a heart whose frequency is lowered by excess K (Fig. 10); I have already pointed out (Crozier, 1924–25) that NaCl and KCl may alter the velocity of toxic action, without disturbing the value of the critical increment; in the case studied (data of Koltzoff, 1913) the critical increment was also that associated with respiration (16,800). One other case of toxic action in the presence of salts (Dernoscheck, 1911) shows that the concentration of NaCl determines the velocity of death of *Daphnia* at constant temperature, but the value of μ is here 11,000.

3. The frequency of rhythmic movements in strips of rabbit intestine, dependent on oxygen, provides μ = 16,150 between 30° and 40°; from 20–30°, it is lower (8,000±); the curves are parallel for different levels of the intestine (data of Taylor and Alvarez, 1917).

4. Gray (1923–24) shows that ciliary activity and O_2 consumption in the gill epithelium of *Mytilus* are in constant proportion. I have pointed out that the critical increments are practically identical (1–15°, 16,700; and 15–34.5°, 11,590; Crozier, 1924–25).

5. The rate of regeneration of polyps in *Tubularia* (Moore, 1910) gives μ = 16,800 (14–23°); oxygen is necessary for the regeneration (Loeb, 1891).

6. The velocity of the latent period process in strips of turtle ventricle (Snyder, 1911) yields μ = 16,170 (14–30°) and μ = 24,750 (0–14°).

7. The velocity of the latent period process in the contraction of strips of cat bladder (Stewart, 1900) gives μ = 17,000.

8. The velocity of conduction of the heart wave in *Ascidia atra* (Hecht, 1917–18) gives μ = 16,400.

9. The velocity of the process underlying first order periods in the discharge of the electric organ of *Malapterurus* (Koike, 1910) shows μ = 16,500.

10. The velocity with which the action current in frog spinal nerve rises to a maximum (Gasser and Erlanger, 1922) gives μ = 16,180.

11. The activities of the nerve net in the body of the colonial cœlenterate *Renilla* (Parker, 1920) and in the foot of the gasteropod *Limax* (Crozier and Pilz, 1923–24) yield μ = 11,200 and 16,100 (*Renilla*) and μ = 10,700 (*Limax*).

12. The velocity of the process underlying the first oscillation in the electromyogram of frog gastrocnemius (Judin, 1923) gives μ = 11,000 (2.4–15°) and μ = 16,000 (15–20°).

13. The frequencies of respiratory movements in the aquatic *Libellula* larva (Babák and Růcek, 1909) give μ = 11,000 (above 15°) and 16,460 (below 15°); and in *Dixippus* μ = 16,800 (von Buddenbrock and von Rohr, 1922–23) and in another series of measurements, 11,500; the frequency of the movements is dependent on the oxygen tension; the rates of breathing movements in the fish *Barbus* (Kuijper, 1907) give a value of μ agreeing fairly well with these (15,000 or higher).

14. The velocity of the "presentation time" processes for geotropic and phototropic curvatures in *Avena* yield respectively $\mu = 16{,}300$ and $\mu = 16{,}500$ (Fig. 11).

15. The velocity of elongation of the radicle of *Pisum*, under certain conditions of experimentation, yields 16,450 (Leitch, 1916; Fig. 13).

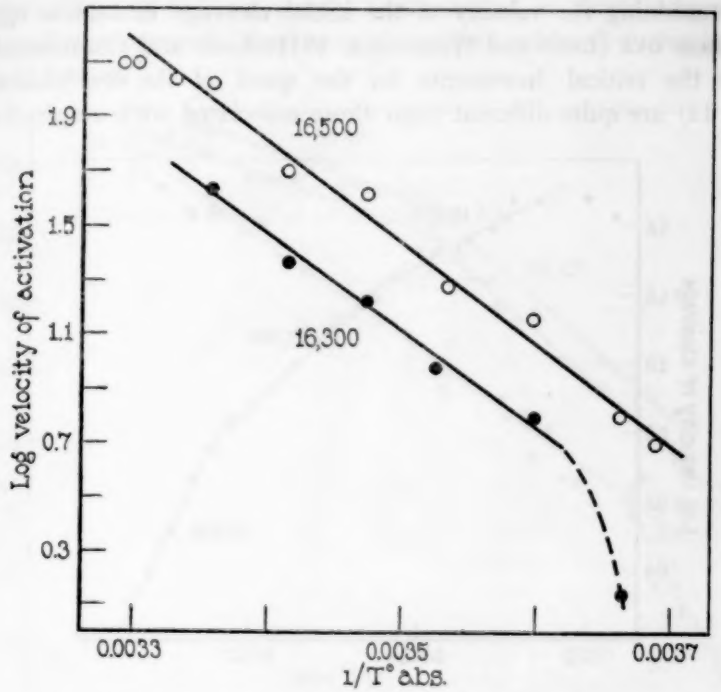

FIG. 11. The velocities of the activation process occupying the "presentation time" for geotropic response (lower graph, data of Rutgers, 1910) and for phototropic response (de Vries, 1913)in *Avena*.

It is difficult not to believe that these various processes yielding sensibly identical critical thermal increments involve some common fundamental process, of a (relatively) simple character. The evidence is strong that the basic reaction is a catalyzed respiratory oxidation. Organic activities which show quite different critical increments we may safely assume not to be controlled by the same reaction. The relations of cell division and growth to temperature are in this connection especially interesting. There is but scant indication that oxidative processes of the sort involved in measurements of respira-

tion have anything to do with the control of the velocity of cell division, differentiation, or growth. Loeb (1913) recognized that by the indications afforded by temperature coefficients the velocity of respiratory oxidations was shown not to be the independent variable in determining the velocity of the initial cleavage in *Arbacia* eggs. For these ova (Loeb and Wasteneys, 1911; Loeb and Chamberlain, 1915) the critical increments for the speed of the first cleavage (Fig. 12) are quite different from those associated with respiration.

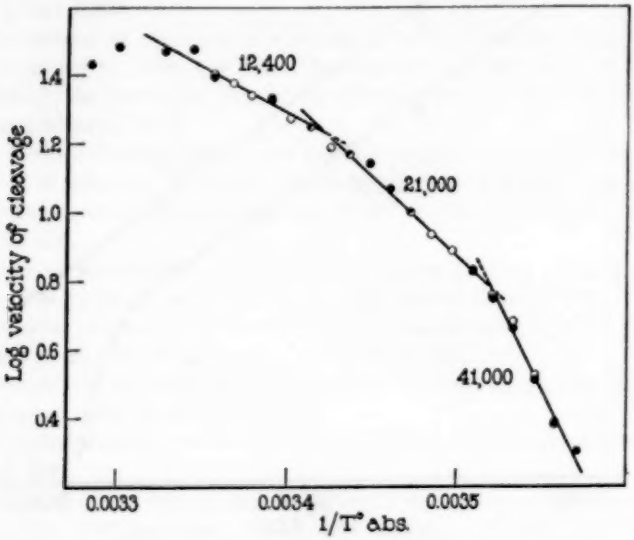

FIG. 12. Velocity of segmentation (first cleavage) in *Arbacia* ova; black circles, data of Loeb and Wasteneys (1911); white circles, data of Loeb and Chamberlain (1916).

For velocity of segmentation in frog ova Krogh's (1914) data yield (below 13.5°) $\mu = 22{,}600$ and (above 13.5°) $\mu = 10{,}200$. This method of inquiry, it may be emphasized, is able to differentiate between "growth" due to different causes. Miss Leitch (1916) measured the early growth of the radicle of *Pisum* at different temperatures, in one series of experiments extending over periods of 22.5 hours and in a second series extending over periods of $\frac{1}{2}$ hour. The first series thus included the hours of darkness, and the daily rhythm of cell division in plants would suggest that the limiting condition for the

rate of elongation of the radicle would thus be expected to differ in the two cases. One is therefore not surprised to find (Fig. 13) that while the increment 16,450 is associated with the rate of elongation during brief (daytime) periods, the increments 20,300 and 8,170, intersecting at 15°, are clearly demonstrated in three independent sets of measurements based upon elongations achieved during periods of 22.5 hours.

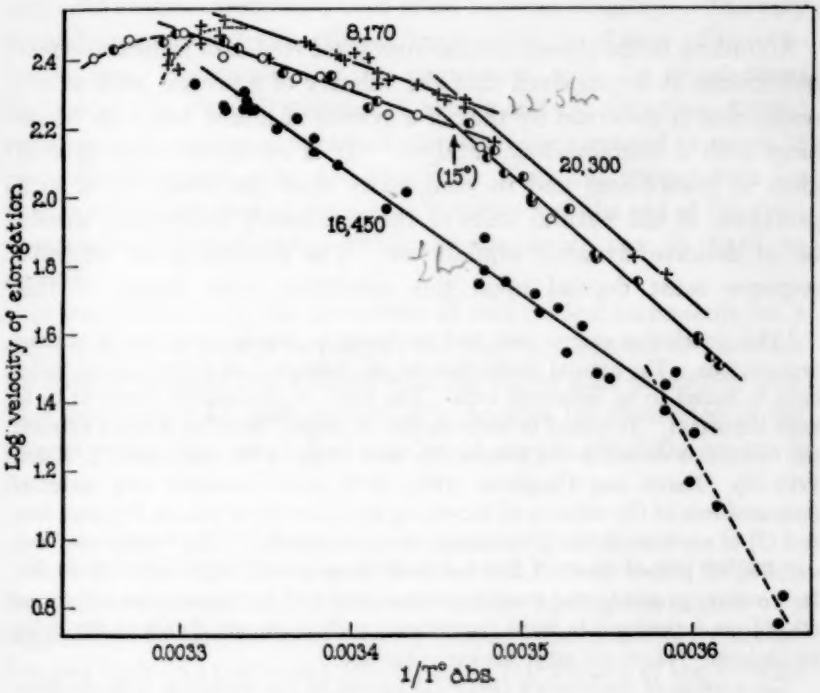

FIG. 13. The rate of elongation of the radicle of *Pisum*—lowest curve, period of 0.5 hour; upper curves, three series of data from measurements of elongation during period of 22.5 hours (Leitch, 1916).

Thus the critical increments for oxygen utilization and for CO_2 production do not appear to be characteristic for measurements of growth (Figs. 1, 12, 13). Since development must depend upon synthetic processes, it would indeed be very surprising to discover any such relation. Yet some have not hesitated to argue, in the absence of suitable quantitative evidence, that the most rapidly "metabolizing"

(*i.e.* in practice, *respiring*) portion of an embryo must control and direct the differentiation of the whole (*cf.* Child, 1915). It is possible that these questions may be best examined by the method of critical increments suggested in the present papers, for the outcome of the inquiry seems to give a means of characterizing protoplasmic processes which involves no destructive interference with the living material.

IV.

According to the theory arising from these studies of critical thermal increments, it is conceived that the velocity of a process such as cell respiration is governed by that of a system of linked reactions which have each a characteristic catalyst.[7] It is consistent with general ideas of protoplasm and its complexity that the velocities of such processes, in the natural state of the organism's adaptation, should be in delicate dynamic equilibrium. The possibility of adaptive response must depend upon this condition. One aspect of this

[7] This conclusion cannot very well be tested by the investigation of enzyme preparations. The natural mechanism for the formation of active enzyme molecules is bound to be interfered with. The mode of preparation seems to be in itself important. It cannot be assumed that in pulped tissues or in tissue extracts the conditions of action are comparable with those in the organism (*cf.* Rahn, 1915–16). Harris and Creighton (1912, 1915) have published two series of measurements of the velocity of enzymatic reduction (1) of soluble Prussian blue and (2) of oxyhemoglobin (determined spectroscopically). The "reductase" was supplied by pulped liver. I find for these series μ = (1) 9,580 and (2) 13,400. It is entirely probable that conditions (of acidity and of "heterogeneity," among others) are introduced in these experiments such as do not obtain in the living protoplasm. There are other similar instances.

The average of Amberson's (1921–22) figures for the oxidation (dehydrogenation) of luciferin by luciferase with production of light, gives μ = approximately 25,000; the system is known to be very sensitive to OH ions, and is not affected by KCN (Harvey, 1920); the *controlling* reaction appears in this case to be an hydrolysis. On the other hand data from Heymans and Moore (1923–24) suggest that the exhaustion of luminescence in slime isolated from the medusa *Pelagia noctiluca* may be due to a process essentially similar to the respiratory oxidations. For two series of determinations in 0.6 M $MgSO_4$, μ is found approximately 10,500 and 16,600; for another series of measurements, in 0.9 M NH_4OH, μ is about 15,500; the data are irregular. The further investigation of bioluminescence from the standpoint of critical increments might permit a decision as to the chemical similarity of the mechanism of light production in different animals.

dynamic balance is believed to be revealed by the occurrence of unmistakable shifts in the nature of the relationship between temperature and velocity, in the neighborhood of a certain (significantly "normal"?) temperature. This frequently occurs at about 15°. To what extent and in what way this temperature is chemically an "inevitable" critical temperature for protoplasm cannot be decided until poikilothermous forms naturally adjusted to very warm and to very cold environments have been more fully investigated. The very general occurrence of this critical temperature, in tissues of invertebrates, frog, mammals (*e.g.* in connection with the spontaneous rhythm of the excised intestine of rabbit and cat (Magnus, 1904)) encourages the notion of its association with some general property of protoplasm—though why 15° should be a critical temperature for objects so far removed as the rabbit intestine and the egg of the clam *Cummingia* (*cf.* Heilbrunn, 1924), or the sweet pea, is hard to explain.

In connection with the occurrence of two critical increments for a single activity it should be pointed out that in a number of instances the lesser increment is associated with the higher zone of temperature. This fact cannot be used theoretically, because (as illustrated in the present paper) there are a good number of cases in which the reverse is true.

V.

It should be very desirable to obtain some hint as to the nature of the catalysts concerned. While admittedly having the status of a suggestion, the outcome of inquiry in this direction is distinctly attractive and leads to a consistent provisional interpretation of the velocity of respiration as affected by temperature.

Biological oxidations are now believed to involve quite frequently, if not generally, a chemical mechanism of dehydrogenation (Meyerhof, 1918, 1923, *a* and *b*; Hopkins, 1921; Wieland, 1922). The relation between temperature and the time of reduction of methylene blue by bacteria in the presence of Na succinate has recently been measured under controlled conditions by Quastel and Whetham (1924), although no analysis of the result is given by them. The reduction system in this case includes the reaction

$$\text{Succinic acid} + \text{methylene blue} \rightleftharpoons \text{fumaric acid} + \text{leucomethylene blue.}$$

This system is thought to have close analogy with the reduction system in fresh muscle and other tissues (Hopkins, 1921), which Hopkins has shown to be of very general occurrence.

I have calculated the velocities of reduction from the determinations plotted by Quastel and Whetham, and the outcome is given in Fig. 14. The critical increment is $\mu = 16{,}700$. So striking an agreement with the values of μ already given for tissue respiration is dis-

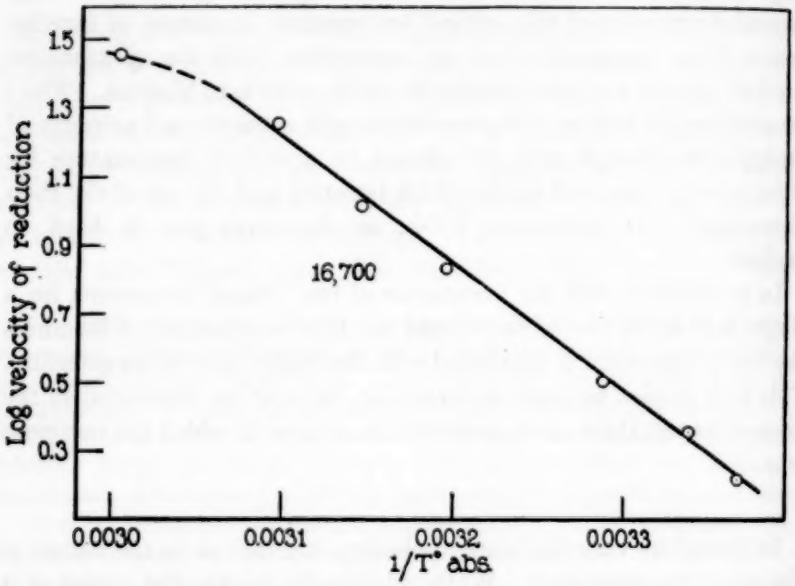

FIG. 14. The velocity of anaerobic reduction of methylene blue by bacteria in presence of Na succinate as source of H (data of Quastel and Whetham, 1924); above 50° destructive effects are evident.

tinctly encouraging. Quastel and Whetham (1924) have further shown that the velocity of reduction at constant temperature is a function of the hydrogen ion concentration. It is of interest that the reduction does not occur at acidities greater than pH 5.0 (or less); according to Rice (1923) the catalytically neutral point for water is pH 5.6. Between pH 6 and about 7.5 I find from their figures that the logarithm of the velocity of reduction is directly proportional to the pH. These two facts are consistent with the view that the veloc-

ity of the reduction is a function both of the hydroxyl ion concentration and of the temperature, and make it possible to suggest why in biological oxidations the increments 11,500 and 16,800 are both found. The assumption is, that in a reduction system of the kind identifiable in many living tissues the velocity of oxidation reduction is determined by two catalytic processes, one of dominant speed throughout in some cases, the other in other cases, while in still different instances the relative velocities of the two processes "intersect" at a certain temperature; the value 11,500 ± is rather definitely associated with hydroxyl ion catalysis (Rice, 1923).[8]

A further point concerns the possibility that the values $\mu = 16{,}700$ ± and $\mu = 16{,}100$ ± are really distinct. So small a difference (3.6 per cent) is scarcely outside the limit of error of determination, and the chemical environment may be sufficiently different to produce a real variation of this magnitude. Yet the fact must be given due emphasis that for the oxidation of Fe'' to Fe''' (by $KClO_3$, in acid solution) Noyes and Wason (1897) found $\mu = 16{,}180$. The earlier experiments of Hood (1885) on the oxidation of $FeSO_4$ yield a value of μ concordant with this. The significance of this possibility lies in the rôle of iron as respiratory catalyst. Slator (1903) found for the reaction between chlorine and benzene $\mu = 16{,}880$, when catalyzed by $FeCl_3$; with other metal ions as catalysts μ differed considerably from this. It is of course possible that in different cases two respiratory mechanisms may exist with critical increments respectively 16,100 ± and 16,700 ±. Note that in the respiration of echinoderm eggs (Fig. 5) with which (Warburg, 1914; Warburg and Meyerhof, 1913) the theory of catalysis by iron is intimately connected, $\mu = 16{,}140$; while for the deoxygenation of hemoglobin (by CO) I have calculated from the data of Hartridge and Roughton (1923, *b*) $\mu = 16{,}525$ (Crozier, 1924–25).[9,10] The velocity of *oxygenation* of hemoglobin (Hartridge and Roughton, 1923, *a*) I find to yield $\mu = 25{,}650$ (acid

[8] It is of interest that the oxidation of NaH_2PO_2 by palladium black (Sieverts and Peters, 1916) yields $\mu = 11{,}000\pm$.

[9] These experiments are important as demonstrating that the method of critical increments is applicable to very rapid reactions.

[10] The reduction of $KMnO_4$ by gaseous CO (Just and Kauko, 1913) provides $\mu = 11{,}060$.

solution) and 20,880 (alkaline solution); these increments are not surprising in view of the rôle of the hydrogen ion (Hartridge and Roughton, 1923, *b*) in this process.

The fact is remarkable, and important for my argument, that a somewhat exhaustive search through published data has failed to show a single clearly understood reaction, other than those cited, in which μ = 16,000 to 17,000. The purely chemical data are much more fragmentary than one could wish, but suffice to show that many oxidative reactions provide μ = as low as 5,200. The exceptional

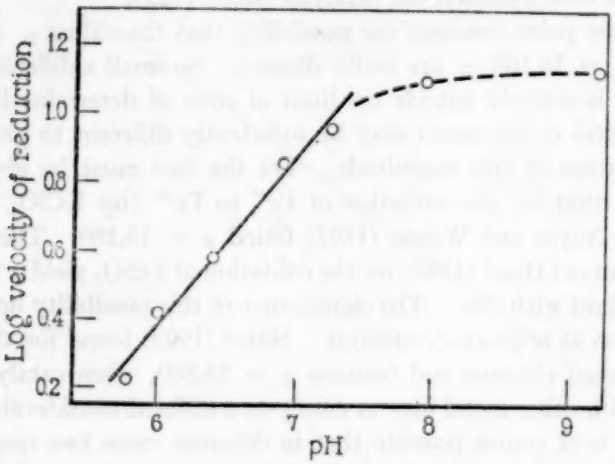

FIG. 15. The velocity of reduction of methylene blue by bacteria is proportional (pH 6 to 7.25) to a fractional power (0.5±) of the hydrogen ion concentration (from data of Quastel and Whetham, 1924).

case of castor lipase acting on triacetin (μ = 16,700) is not well enough understood to disturb the general conclusion. The data of Drushel and Dean (1913) and of Dean (1914) lead to μ = 16,500 ± for the hydrolysis of ethereal salts by HCl, but lower values are obtained from experiments by other workers. The oxidation of iodine, in acid solution, in different processes yields 15,850 or 18,550 (in the dark); the reaction between CHI_3 and acid (μ = 15,400 ±) and the decomposition of trichloroacetic acid (μ = 16,230, in the dark), like the first two reactions cited, may be catalyzed by iron (*cf.* Dushman, 1904; Plotnikow, 1907, 1910–11; Dhar, 1917; Banerji and Dhar,

1924). (It is doubtful if the actual measurements, for any of these processes, are of more than moderate precision.)

Conceivably, iron is associated as promotor catalyst with the functioning of the sulf-hydryl reduction-oxidation system (Meyerhof, 1918, 1923, *a*, and *b*; Hopkins, 1921); perhaps its place may be to some extent taken by other metals (Cu, Mn, Va). The implication of iron in such processes seems definitely established, but the erratic manner in which these oxidations may or may not be inhibited by cyanide is in itself sufficient to suggest that the mechanism is not altogether a simple one (*cf.* also Baudisch and Welo, 1924). For purposes of orientation in planning further experiments one may be required to choose between two alternative interpretations: either the different critical increments associated with respiration are connected with different processes involved in the formation of active molecules of a respiratory catalyst, or else with different steps in the history of the respiratory substrate (glycogen). Each of these alternatives has certain advantages, and if it is recalled that temperature characteristics akin to those of hydrolytic processes are also encountered (under inanition) it can be assumed that in the general analysis of respiration and of processes dependent thereon *both* hypotheses may be useful and not mutually exclusive.

SUMMARY.

1. The critical thermal increments are calculated for respiratory processes (O_2 consumption, CO_2 production) in various plants and animals. They are characteristically found to be of two, possibly three, types: μ = 11,500, and 16,100 or 16,700. The first is commonly encountered above 15°, the second below that temperature, but these relations may be reversed. (The value of μ may be significantly changed in inanition.)

2. For reduction of methylene blue by bacteria, through removal of H from succinic acid, μ = 16,700. This process (Quastel and Whetham, 1924) at constant temperature is a function of the hydroxyl ion concentration. The suggestive identity is pointed out of the critical increment for this reduction phenomenon with that deduced for biological respirations in which a dehydrogenation mechanism is supposed to be of widespread occurrence, and in connection with which

Fe very likely has a catalytic rôle. The action of OH' is believed to be revealed in the value $\mu = 11{,}500$, frequently obtained in connection with respiration.

3. A somewhat lower μ (16,140) is associated with the oxidation of $Fe^{\cdot}$, and may be compared with (1) that of respiration in sea urchin eggs, for which (Warburg) iron is catalyst, and (2) that for some simple reactions in which Fe is known to serve as catalyst; it is not found for oxidative reactions in which Fe is not involved.

4. The bearing of these findings is discussed in relation to the theory of functional analysis of concurrent catalyzed reactions in protoplasm. It is shown that for a number of activities in which the effects of respiration may safely be assumed, the values of the critical increments are consistent with those determined for processes of respiration.

5. The further development of these views may lead to an extremely important method of identifying controlling reactions in undisturbed living matter.

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THE PHOTOTROPIC MECHANISM IN RANATRA.

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(Accepted for publication, September 28, 1924.)

The theory of the phototropic movements of typical insects, as proposed by Loeb (*cf.* Loeb, 1918), has conceived that forced orientation under the influence of light is due to the effect of asymmetrical sensory excitation upon the postures of opposed appendages. Asymmetrical posture of locomotor organs, resulting from forced asymmetrical stimulation of the eyes, was adequately described by Holmes (1905; 1908) and by Garrey (1917; 1918–19) for various arthropods. The respective rôles of the *rapidity of leg movements* and of the *postures of the legs*, during the course of orientation by light, have, however, never been made clear. The fact that an experienced observer (*e.g.* Mast, 1923–24) can at this late date make an inexcusable confusion between *tonic* effects determining limb posture and the *frequency* of leg movements shows that the point here raised is especially worth settling by means of a crucial case.

This particular instance is regarded as crucial because it is possible to determine by inspection the significance of limb posture in effecting orientation. The differential tonic actions of light in the case of the "water scorpion," *Ranatra*, were described some years ago by Holmes (1905). Among other things, Holmes described the "circus movements" of *Ranatra*; unfortunately, the experiments were made with horizontal illumination, and such conditions are unsuitable. If one eye of a *Ranatra* be occluded, or removed, the animal continues for hours to circle toward the remaining eye, *if the insect is in water and is illuminated from above.*

The special point of the experiment is this: During *creeping* on a table top, in air, or on vegetation under water, the opposite legs of each locomotor pair are in alternate phase, the diagonally opposite

legs being in phase; if the two anterior (raptorial) limbs are used (as may be the case) they too work alternately, but the raptorial pair is not closely interconnected nervously with the two pairs of ambulatory legs, these last forming in reality a closely knit effector unit. But in *swimming* under water the legs of a morphological pair work synchronously, in phase; the two locomotor pairs alternate in execution of the propulsive stroke; the anterior raptorial legs, which may be

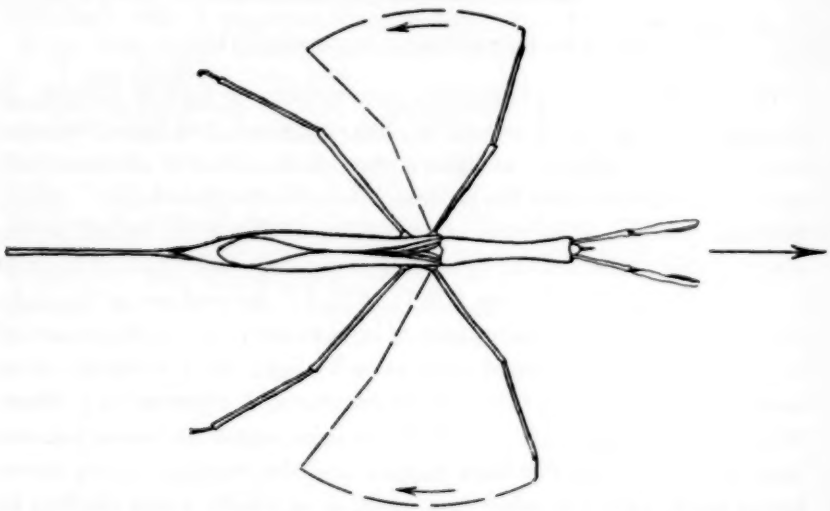

FIG. 1. Showing positions of the locomotor appendages in a swimming *Ranatra* from which both eyes have been removed. The two limbs of a pair move synchronously and in phase. At the conclusion of the stroke (dashed lines) the legs are slightly more extended than at the beginning of the stroke (full lines); as the beat of the anterior legs is concluded the posterior pair are moved to a position comparable to the initial position of the anterior limbs; thus the two pairs move in alternate phase.

used if the insect is in contact with the surface film (or, head down, with the bottom) are used alternately, though during swimming in mid-water they are inactive.

Our observations were made upon *Ranatra americana*. If both eyes of an individual are amputated, and the animal put in a large cylindrical vessel of water in a dark room, with a single point source of light above the aquarium, the *Ranatra* stays near the surface of the

water and moves aimlessly. The body remains horizontal. But if this be repeated using a *Ranatra* from which *one* eye has been removed there is witnessed a very perfect circus movement response. The positively phototropic animal swims in a compact circular path, turning continually toward the side carrying the one functional eye. If a white surface is at the bottom of the aquarium the *Ranatra* turns the eye side downward, and swims in a spiral to the bottom of the aquarium, curving always toward the remaining eye. This is continually

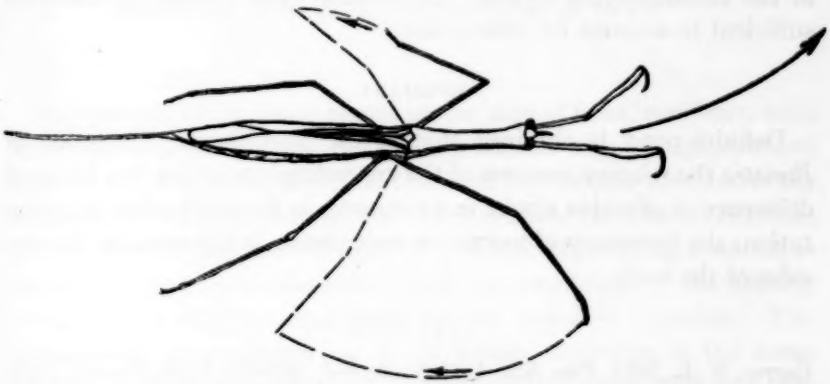

FIG. 2. Asymmetric tonus in legs of a swimming *Ranatra* from which one eye (right) has been removed. The legs on the blind side assume positions comparable to those of the appendages of a completely de-eyed *Ranatra* (Fig. 1). On the eye side the tonus of leg flexors is increased. This results in hydrostatic tilting of the whole body at rest. In swimming the two legs of a pair move synchronously but the greater flexor tonus on the eye side is maintained throughout the execution of the swimming stroke, so that the extension of the left legs during the propulsive motion (arrows) is less than on the relatively atonic right side. The animal accordingly swims continuously in a circle toward the eye-bearing side.

maintained for long periods. With a dead-black bottom the *Ranatra* stays at the top of the water, the eye side tilted slightly upward, and with the body almost horizontal in the surface film. Preliminary experiments have shown that the diameter of the circle in these circus movements is a function of light intensity and of wave length of light.

The important part of the observation is, that during these long continued acts of maintained orientation the two legs of each pair

work synchronously and in phase. The notion that orientation is due to a relatively greater frequency of leg movements on one side of the body is definitely disposed of. But the relative *postures* of the limbs on the two sides of the body, during movement, are quite different. The leg movements are so slow (one beat per second at 16°C.) that these facts are easily demonstrable to anyone witnessing the act of phototropic orientation, and accurate sketches can be made of the postures of the appendages. The essential facts are illustrated in the accompanying figures. Differential limb posture is therefore sufficient to account for orientation.

SUMMARY.

Definite proof is obtained that in the phototropic orientation of *Ranatra* the relative postures of the appendages, resulting in a bilateral difference of effective stroke in swimming, is the mechanism of orientation; the frequency of locomotor movements is the same on the two sides of the body.

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SUPPRESSION OF PHOTOTROPIC CIRCUS MOVEMENTS OF LIMAX BY STRYCHNINE.

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(Accepted for publication, September 23, 1924.)

I.

Phototropic circus movements of the slug, *Limax maximus*, with illumination constant, are affected in a curious way by temperature. Above 15°C. the amplitude of circling is an exponential function of the temperature, and the concomitant rates of linear progression decrease as the temperature rises. Below 15° the rate of creeping is an exponential function of temperature, while the amplitude of the turning posture is inversely proportional to the speed of creeping. The temperature characteristic, μ of Arrhenius' equation, is the same (16,800) for both amplitude of circling posture (above 15°) and rate of linear movement (below 15°). The interpretation proposed (Crozier and Federighi, 1924–25) for these facts turned upon (*a*) the central nervous partition of the impulses proceeding (1) to the creeping mechanism and serving to release pedal waves, and (2), to the parietal muscles concerned with orientation; and (*b*), upon the dynamic efficiencies of the two sorts of effector response. The validity of this conception may be tested by attempting to separate in an analytical way the central mechanisms respectively having to do with control of the pedal organ and with the maintaining of differential bilateral tonus. It is shown in the present paper that such separation is possible.

The most delicate method of accomplishing this purpose is to utilize the selective effects of neurophil drugs. It was pointed out (Crozier and Federighi, (1924–25) that under certain conditions a *Limax* into which strychnine solution had been injected will creep rapidly in a straight line, even though one eye-tentacle has been removed and the animal is illuminated from above; under these

circumstances a non-strychninized *Limax* circles continuously toward the de-tentacled side (Crozier and Cole, 1922; Crozier, 1922–23). But this proof of interconnection between the nervous mechanisms of creeping and of turning is incomplete; it might be that in these tests mere rapid creeping mechanically hindered circling. We have therefore attempted to make the demonstration more convincing.

II.

Since the time-course of the sequence of events due to the injection of a *Limax* with strychnine depends upon the amount and the concentration of the drug solution, it is desirable to demonstrate the degree of specificity manifested in the effects of this substance. From this standpoint the actions of eleven other neurophil substances have been examined. The reactivity of the head region is markedly enhanced following injection of strychnine, camphor, nicotine, phenol, or picrotoxin. Extension of the body is increased by strychnine, camphor, or picrotoxin. But the general effects of each substance are specific. Pilocarpine, for example, is peculiar in producing an almost complete loss of tone in the body musculature; the slug seems to collapse; when picked up by grasping at any point with forceps, the animal is like a sac, the internal organs flowing to the lowest point. Strychnine is peculiar in that it alone of substances tested evokes "reversal of inhibition." Thus if the caudal keel be rubbed gently, in a normal *Limax*, the tentacles are extended and creeping is promptly initiated; but under strychnine this sort of stimulation causes forcible retraction of the head structures, the rapid forward extension of the mantle, and even the folding of the mantle ventralward, "protectively," over the anterior end of the foot.

Effects of the general type thus shown to be more or less characteristic of strychnine action are known to be produced by it in other mollusks (Frölich, 1910; Moore, 1916–17; *Chromodoris*, Crozier and Arey, 1919; *Onchidium*, Crozier and Arey, 1919–20; *Chætopleura*, Crozier, 1919–20). Since the central effect of strychnine may in at least some instances be understood to depend upon the elective excitation of particular groups of motor neurones, and since, in *Limax*, moderate doses do not interfere with local reactions of the integument nor with the mechanism of locomotion, its use is indicated as most suitable for the present purpose.

III.

Preliminary experiments showed that at a certain stage in the progress of strychninization the slugs no longer responded to light. This effect may persist for a number of hours. It is probably due to an influence upon essential normal central nervous processes, because non-injected normal individuals may occasionally fail to respond to light for periods of a day or longer; temporary phases of non-reactiveness to photic excitation may be purposely brought about by certain types of feeding.

Experiments were begun 2 hours after the injection of 0.5 cc. of saturated strychnine hydrosulfate solution. The slugs had 2 days before undergone the amputation of one eye-tentacle. Circus move-

TABLE I.

Effect of Strychnine in Suppressing Phototropic Circus Movements of Limax; 4 Sets of Experiments (23 Animals), Tested 2 to 3 Hours after Receiving Injection of 0.5 Cc. Saturated Strychnine Hydrosulfate.

Temperature.	Circus movement, degrees per cm.	Velocity of creeping, cm. per min.
°C.		
29.5	10.8	0.065
29.6	11.75	0.070
29.8	18.17	0.077
30.1	17.88	0.103
Control, normal animals..29.8	82.86	0.053

ment tests were carried out at about 29.7°. A high temperature was chosen because the normal phototropic deflection, measured as degrees deflection per cm. of path, is increased the higher the temperature (Crozier and Federighi, 1924–25). Opportunity is thereby given for the enhancement of any circling tendency. Moreover, with doses of strychnine of the concentration used, the animal creeps more slowly than do non-injected, non-circling animals; so that with these individuals the expression of orientation cannot be mechanically hampered by the vigor of the creeping movement. The light intensity and the other conditions of the experiment were exactly as described in our previous paper (Crozier and Federighi, 1924–25). Non-injected animals of similar size and history were used as controls.

The table summarizes the results of these tests.

In these tests there is detected a slight residuum of phototropism, and it will be noticed that the amplitude of turning and the velocity of creeping are increased by even a small increase in temperature. But the amount of circling is only about one-fifth as great as that of non-strychninized animals. Correspondingly, the rate of locomotion is higher than with the non-injected, circling individuals.

The practical abolition of phototropism was also demonstrated by means of experiments involving horizontal illumination with lateral light. The results are consistent with those already described, but cannot so easily be expressed in a quantitative way.

IV.

CONCLUSION.

By injection with strychnine the phototropic circus movements of the slug *Limax maximus* may be suppressed, its phototropism abolished. The creeping activity of the foot is not in any essential way interferred with. Strychnine produces in *Limax* central nervous effects of the sort associated with its characteristic action. Hence, although an effect of the drug upon photoreceptors cannot be definitely excluded, the experimental result is held to demonstrate that in orientation during circus movements there occurs central "competition" between impulses resulting (1) in the release of pedal waves and (2) in the maintenance of a turning posture.

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THE EFFECT OF VALENCY OF CATIONS AND ANIONS ON NEGATIVELY AND POSITIVELY CHARGED RED BLOOD CELLS.

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(Accepted for publication, September 27, 1924.)

It is a well established fact that the ion of an electrolyte which acts on the charge of a suspended particle is that one which bears a charge of the opposite sign to that of the particle. Thus the negatively charged particles of a collodion suspension have their charges reduced and may be flocculated by the cation of an added salt, while with the positively charged particles of a copper sol the anion is the active ion.

The efficiency of the active ion in producing its effect on the charge of a particle, or on the stability of the suspension, varies with its valency. Exact quantitative relations have not, however, been established, as widely varying results have been obtained by different observers in their attempts at an experimental determination of the correlation. A summary of this work may be found in Burton's monograph (1).

In the earlier investigation we have studied the effect of electrolytes on red blood cells suspended in isotonic sucrose (2). Under these conditions the cells are negatively charged and the changes in the P.D. of the cells which occur as the electrolyte is added depend in part on the valency of the cation. The effect of valency may, however, be masked by the action of H ions in the suspending fluid, and in the case of hydrolyzing salts this becomes a factor of considerable importance.

In the present study we wish to contrast the effect of the valency of the cation on negatively charged cells with the effect of valency of the anion on positively charged cells. It is possible to obtain cells positively charged by increasing the H ion concentration of the

suspending fluid. Above the isoelectric point of the cells, using this term in the sense of Michaelis, their original negative charge is reversed. Now under such conditions Loeb (3) has shown that proteins combine not with the cation of the electrolyte present, but with the anion, and Coulter (4) has demonstrated that the same principle holds for red blood cells. There seems, therefore, strong *a priori* evidence to support the hypothesis that the electrokinetic effects produced by the anion on positively charged red cells will be analogous to those produced by the cation on the negatively charged cells.

Methods.

Rabbit cells washed as free as possible of electrolyte with isotonic sucrose and suspended in the same fluid were used in all the experiments. The electric charge of the cells was determined with a Michaelis cataphoresis chamber and the results expressed in millivolts by means of the usual formula. The suspension of positively charged cells was produced by adding HCl to give a concentration of M/250 in the total volume of suspension.

The Effect of the Cation.

In studying the action of the cation on the two types of cells three salts, NaCl, $NiCl_2$, and $AlCl_3$, were used with cations which are respectively monovalent, bivalent, and trivalent, the anion being the same in all cases. Two sets of series of tubes were prepared, one containing negatively charged cells in isotonic sucrose and the other positively charged cells in the same fluid containing M/250 HCl. To both sets of tubes were added increasing concentrations of the three salts and the P.D. of the cells determined. As has been shown (2), the P.D. of the negatively charged cells in a weak concentration of electrolyte is not a permanent one. The curves that are illustrated are those of the "immediate charge" as these alone give a full demonstration of the effect of the added electrolyte. With the positive cells the charge is permanent, as there is sufficient electrolyte in the form of HCl to stabilize the charge.

Fig. 1 illustrates the cation effect of the different salts on the two types of cells. Above the isoelectric line the charge is negative,

below, it is positive. Valency effect of the cation is evident in the action of the salts on the negative cells. NaCl produces a slow depression of the charge which reaches an isoelectric point at a

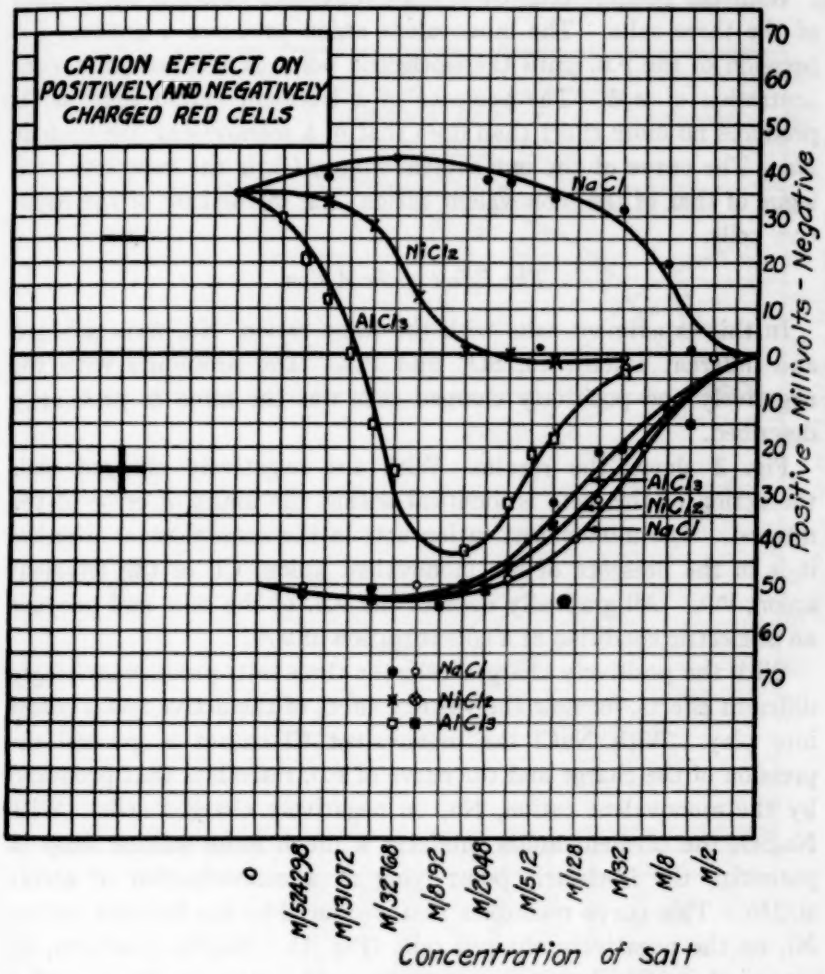

FIG. 1.

concentration of M/4. $NiCl_2$ with a bivalent cation, produces a much more sudden drop in the P.D. of the cells so that the isoelectric point is reached at a concentration of M/4,000. $AlCl_3$ with a triva-

lent cation causes a sudden fall in P.D., the isoelectric point being reached at M/131,072; the charge is then reversed and the typical trivalent ion effect then follows.

With the positive cells there is no difference between the actions of the three salts. The monovalent anion produces a gradual depression of the P.D. until the isoelectric point is reached at a concentration of M/4. The presence of a trivalent cation such as Al produces no more effect than does that of a monovalent one such as Na. The curve of the monovalent anion, Cl, is the complete analogue of that of the monovalent cation, Na, in the case of the negative cells.

The Effect of the Anion.

In this experiment salts with the same cation, Na, were selected and different anions, Cl, SO_4, and PO_4. The procedure with the negatively and positively charged cells was the same as previously described.

Fig. 2 shows the results. With the negatively charged cells the action of the salts is identical within the limits of error of the method. The monovalent cation acts in the same manner whether it is in the presence of the monovalent anion, Cl, or the trivalent anion, PO_4. All gradually depress the P.D. of the cells and produce an isoelectric condition at a concentration of M/4.

With the positively charged cells the three salts produce strikingly different effects, for now the valency effect of the active anion comes into play. With NaCl the monovalent Cl causes a gradual depression of the charge and the curve of P.D. resembles that produced by the monovalent cation, Na, on negatively charged cells. With Na_2SO_4 the bivalent anion produces a much more sudden drop in potential, the isoelectric point lying at a concentration of about M/256. This curve resembles that produced by the bivalent cation, Ni, on the negatively charged cells (Fig. 1). Na_3PO_4 produces, by means of its trivalent anion, a more sudden drop in charge and a reversal of charge at M/512. It resembles, therefore, the curve of P.D. produced by the trivalent Al cation on the negatively charged cells (Fig. 1).

Hydrogen Ion Concentration and the Observation of the Effect of Valency of Ions of Hydrolyzing Salts.

Extensive investigations have been made in attempts to determine the exact relation between the effect of an ion and its valency. In

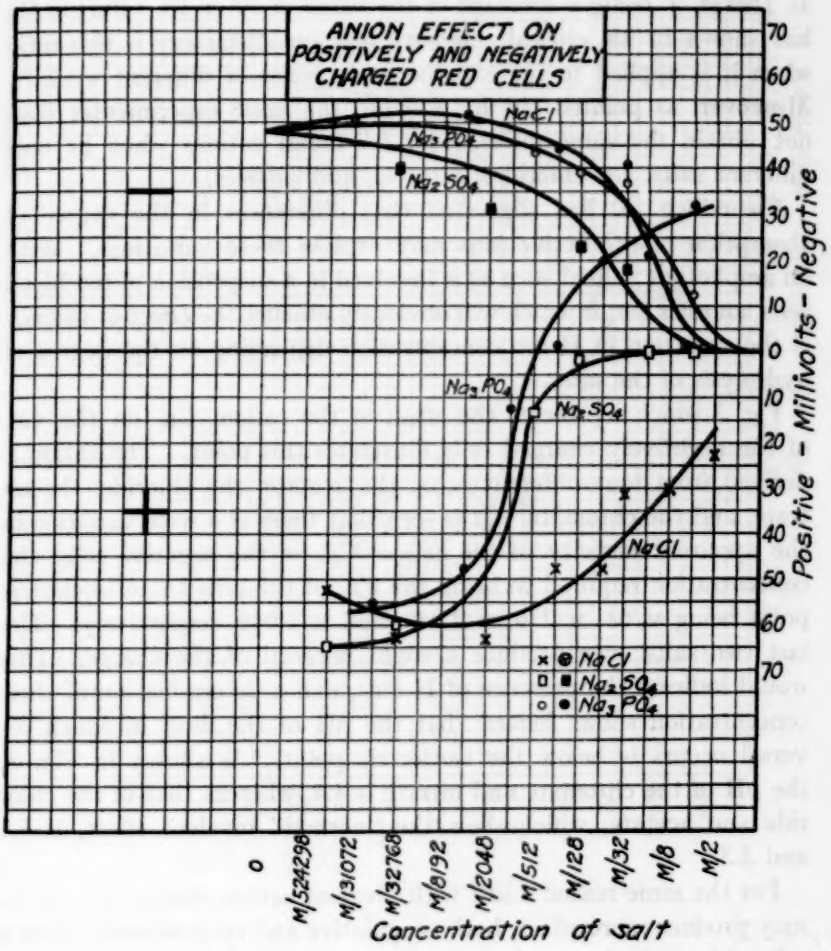
ANION EFFECT ON POSITIVELY AND NEGATIVELY CHARGED RED CELLS
NaCl
Na3PO4
Na2SO4
Na3PO4
Na2SO4
NaCl
× ⊛ NaCl
□ ■ Na2SO4
○ ● Na3PO4
70 60 50 40 30 20 10 0 10 20 30 40 50 60 70
Positive - Millivolts - Negative
0 M/524298 M/131072 M/32768 M/8192 M/2048 M/512 M/128 M/32 M/8 M/2
Concentration of salt

FIG. 2.

most cases the effect studied was the coagulating value of the salt, which, with suspensoid colloids, at least, is largely determined by

the electrokinetic action of the ion. The results of these investigations have been very unsatisfactory so far as determining any quantitative relationship. One of the earliest of such attempts is the Schulze-Linder-Picton law of coagulation, which states that the coagulative value of monovalent, bivalent, and trivalent ions is as $1:x:x^2$, x benig a constant of the value of 30 or 40. Burton (1) has shown in his critical summary how unsatisfactory is this ratio when it is applied to the experimental results of different workers. Moreover, as pointed out by Burton, the same experimenter does not obtain the same series order for metal cations when he uses different salts, *e.g.* chlorides, nitrates, and sulfates.

Freundlich (5) has suggested that differences in the degree of absorption of the active ions may explain these variations. With an amphoteric colloid such as is involved in a suspension of red blood cells, another factor which will obviously obscure the effect of valency is the variation in H ion concentration depending on the degree of hydrolysis of the salt.

Fig. 3 which represents the effect of the cation, Ca, on the P.D. of the negatively charged cells, illustrates this point. The cation is derived from four different salts, the acetate, the chloride, the nitrate, and the chromate. It is seen that there is a wide variation in the apparent activity of the cation, Ca, on the negative cells, the concentration required to bring the P.D. of the cells to an isoelectric point being M/64, M/256, M/2,048, and M/2,048, respectively. The last two salts even produce a slight reversal of the charge. This would indicate the presence of H ions, and a determination of their concentration shows indeed that the pH of the fluid in which reversal occurs is below the isoelectric point. As shown in Fig. 3, the pH of the chromate and nitrate is 4.4, whereas that of the chloride and acetate, which show the "normal" bivalent effect, is 5.5 and 5.3.

For the same reason a salt with bivalent cation, which hydrolyzes, may produce more effect, both coagulative and electrokinetic, than a salt with a trivalent cation which is not greatly hydrolyzed. Fig. 4 shows the effect on the P.D. of the cells and on the stability of the suspension of strongly hydrolyzed $CuCl_2$ with bivalent cation as contrasted with the weakly hydrolyzed $CeCl_3$ with trivalent cation.

The salt of the bivalent ion is more active than that of the trivalent ion in all respects. Both depress the charge of the cells to an isoelectric point at a concentration of about M/32,000; but the $CuCl_2$

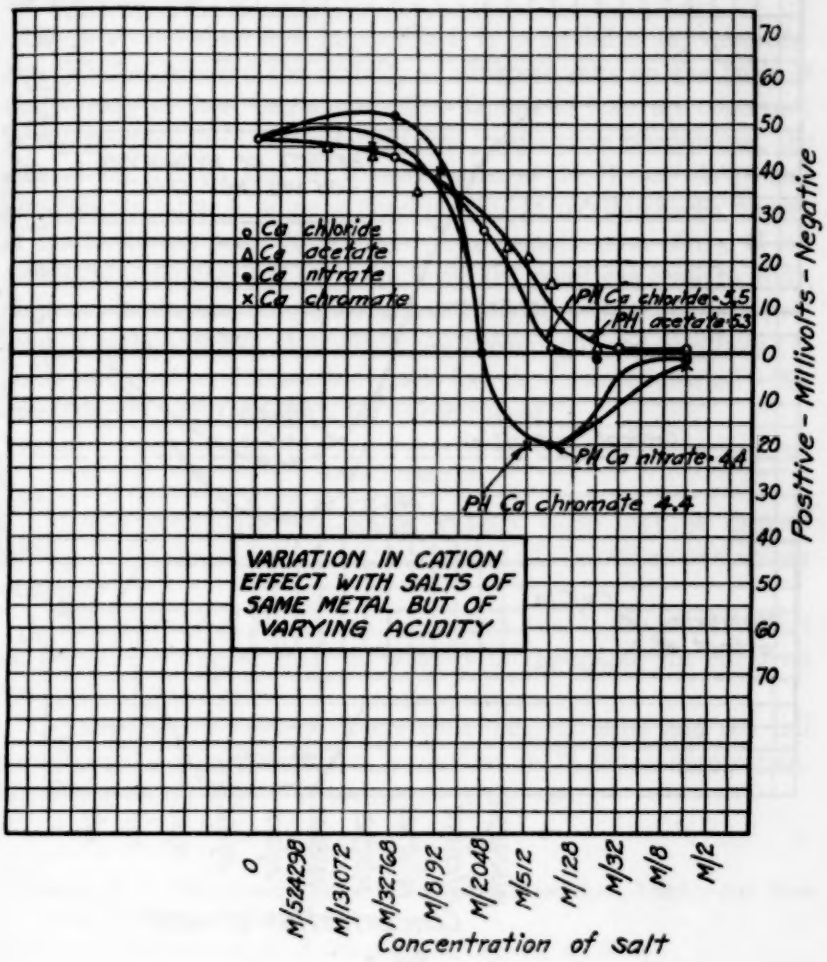

FIG. 3.

produces a strong positive charge which is sufficient to cause a zone of stability, whereas the $CeCl_3$ does not produce a sufficient charge to stabilize the unstable suspension. The difference in the pH of

the same molar concentrations of the two salts is striking. At M/1,000 $CuCl_2$ has a pH of 2.7, while that of the $CeCl_3$ is 4.5.

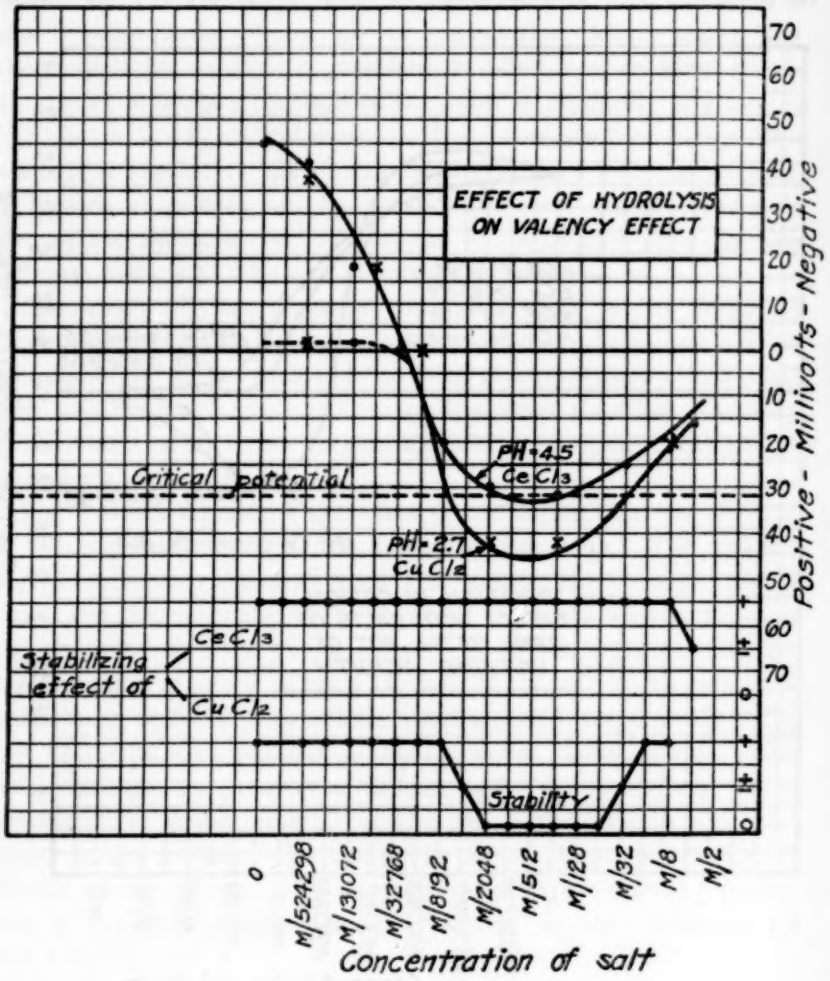

FIG. 4.

DISCUSSION.

When the H ion concentration varies but little as was the case in certain of our experiments (Figs. 1 and 2), the effect of valency may

be satisfactorily demonstrated both with cations and negatively and with anions and positively charged red cells. If the H ion concentration varies too much (Figs. 3 and 4), the effect of valency is entirely obscured.

These results show how impossible it is to establish any quantitative relation between the activity of different salts on the basis of the valency of their ions alone. Two factors at least are concerned in the production of electrokinetic and coagulative phenomena, the general ion activity and the specific action of the H ion. With ampholytes the latter is of great importance. In fact Michaelis' theory of the isoelectric point (6) is based on a consideration of the H ion concentration alone, and the term isoelectric point has come to mean for many biologists, who are concerned largely with amphoteric proteins, a certain degree of acidity. As Freundlich[1] and Svedberg (7) point out, and as Michaelis himself has shown experimentally (8), the activity of all the ions must be considered.

CONCLUSIONS.

1. Under comparable conditions, valency effect may be demonstrated with a suspension of red blood cells and the cations and anions of salts.

2. The valency of the cation determines the degree of the effect on negatively charged cells, the valency of the anion, the effect on positively charged cells.

3. Anomalies in valency effects with different salts and red cell suspensions are in part due to variations in H ion concentration, depending on the degree of hydrolysis of the salt.

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[1] Freundlich (5), p. 784.

THE VISUAL DISCRIMINATION OF INTENSITY AND THE WEBER-FECHNER LAW.

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(Accepted for publication, September 25, 1924.)

I.

Purpose of Work.

1. Differential Sensibility.—The power to distinguish differences in the brightness of objects is an outstanding property of the human eye, and is used constantly in judgments of distance and form. It is not possible, however, to estimate quantitatively the magnitudes of the corresponding differences in sensation. A light may be described as appearing slightly brighter or much brighter than another light, but no numerical values can be assigned to such judgments.

Direct estimation being impossible, it is customary to evaluate differential sensibility indirectly in terms of the minimum change in illumination which can be distinguished as a change in brightness. If I is the intensity to which the eye is adapted, and ΔI the increase in that intensity which is just perceptible, then the ratio $\frac{\Delta I}{I}$ may be considered a measure of the discriminating power of the eye.

The description of the relation between the discriminable threshold ΔI and the original intensity I has had an interesting evolution, culminating in what is currently known as the Weber-Fechner law. According to this law the ratio $\frac{\Delta I}{I}$ is constant within wide limits of intensity, not only for vision but also for other senses. A systematic survey of the literature on the Weber-Fechner law is beyond the scope of the present paper. However, the historical development

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of the Weber-Fechner concept is significant because it illustrates how an idea, on account of its apparent reasonableness, may be absorbed into the body of scientific knowledge and tenaciously held as a general truth, even though it expresses only an extremely circumscribed portion of reality.

2. Statement of Problem.—There exists a body of data which describes accurately and without any preconceived notions the behavior and magnitude of the discrimination ratio $\frac{\Delta I}{I}$ over a great range of illuminations. Clearly, these data represent manifestations of the mechanism in the retina which determines vision. The characteristics of the discrimination ratio, therefore, should find an explanation in terms of this mechanism; and conversely, the capacity of any system to serve as a mechanism of vision may be tested by its ability adequately to furnish the basis for intensity discrimination.

It is the aim of this paper to study the existing facts of intensity discrimination and to suggest an explanation of them in terms of our knowledge of the photochemistry of vision as derived from investigations on the clam, *Mya arenaria*, and on the human eye.

II.

Historical Development of the Weber-Fechner Law.

1. Original Evidence.—The idea embodied in the Weber-Fechner law was described several times independently. The first time seems to have been by Bouguer (1760) as the result of experiments "..... faites pour déterminer quelle force il faut qu'ait une lumière pour qu'elle en fasse disparaître une autre plus faible." Two candles are placed at different distances from a screen, one of them throwing a shadow which is obliterated by the other. Bouguer found that the ratio of the two intensities at this point was $\frac{1}{64}$. He noted cautiously that he had not observed a change in the ratio when the brightness (*vivacité*) of the lights was varied. In developing Bouguer's work, Arago (1858) added that no matter what the ratio $\frac{\Delta I}{I}$ happens to be, it can be reduced still further by keeping the shadow in motion. The

experiments were repeated with a new method by Masson (1845), who reported that though different people give different values of $\frac{\Delta I}{I}$ the ratio is constant for a given person regardless of intensity or color.

Independently of this development, Steinheil (1837) had found that the just perceptible difference in intensity measurable with his newly invented prism photometer was 1 part in 38.

About the same time as Steinheil, and also independently, Weber (1834) discovered for the sense of touch that one could discriminate between two weights if they differed by 1 or 2 parts in 30. Similarly one could just discriminate visually between two lines if they differed by 1 part in 100 regardless of the absolute magnitude of the lines.

Finally the relation was observed by Fechner, from whom it received the impetus which has carried it to the present day. Fechner (1858) had noticed that a slight difference in the shade of a cloud remained perceptible even after the brightness of the cloud was reduced by the interposition of smoked glass. Repetition of Bouguer's experiments with two candles and a shadow showed $\frac{\Delta I}{I}$ to be uniformly $\frac{1}{100}$.

Fechner investigated on this basis the relation between the magnitude of a star and its photometric intensity. Stars had been classified visually into six magnitudes, the brightest being a first, and the dimmest a sixth magnitude star. If each member of this sequence represents a constant fractional decrease in actual intensity, then the arithmetical series of star magnitudes should correspond to a decreasing geometrical series of photometric intensities. Using the then available astronomical data, Fechner described the relation between the magnitude, M, of a star and its intensity, I, by an equation of the form: $M = k \log I + C$.

Such a relation and a similar interpretation of it had already been found by Steinheil (1837) with the first series of measurements of star intensities made with his prism photometer.

Fechner developed the idea of a constant fractional relation between two intensities which produce a threshold difference in brightness. This constant fractional relation he called Weber's law. On the as-

sumption that the difference threshold represents a unit change in sensation, ΔS, he wrote Weber's law as

$$S = k \frac{\Delta I}{I}. \tag{1}$$

On integration this yields $S = k \log I + C$, a relation which Fechner called the psychophysical law, and used as a foundation for his speculations in psychology and philosophy (Fechner, 1860).

2. Criticism.—It was apparent even to Fechner that Weber's ratio is constant only within limits, but he set these limits at the two extremes of the intensity scale. According to Fechner, the upper limit is due to the dazzling effect of high intensities; the lower to the intrinsic light of the retina. To take the latter into account Fechner (1860) introduced its value I_0 into the equation for Weber's law, which then became

$$S = k \frac{\Delta I}{I_0 + I}. \tag{2}$$

It was on this matter of limits that Helmholtz (1866) made the first criticism of the Weber-Fechner law. Helmholtz had experimented with Masson's method, and, contrary to Masson, had found the ratio $\frac{\Delta I}{I}$ to vary at different intensities. Outside daylight gave a ratio of $\frac{1}{150}$ or even $\frac{1}{167}$, whereas interior daylight yielded a ratio of $\frac{1}{117}$. From this Helmholtz concluded significantly that the circumstances "..... which vitiate Fechner's law at the upper and lower limits show their influence under accurate observation in the medium illuminations as well, which naturally does not prevent the law from being a first approximation to the truth."

3. Analysis of Evidence.—In view of Helmholtz's criticism it becomes necessary to consider the nature of the original evidence for the Weber-Fechner law. Bouguer, Arago, and Masson state that the ratio $\frac{\Delta I}{I}$ does not vary with the intensity, but none of them presents any data from which to judge independently the range of intensities investigated or the accuracy of the measurements. Steinheil's measurements are excellent; their range of intensities, however, is about 1

to 4. Weber's own data cover a range of 1 to 16, but his judgments with weights vary between 1 and 2 parts in 30. Fechner states that his experiments, conducted by Bouguer's method, yield a constant ratio of $\frac{1}{100}$ for a range of 1 to 40 units. However, a careful analysis of Fechner's method and data by Aubert (1865) shows clearly that, since it is impossible to measure the position of a candle flame with the necessary precision, the results are reliable only over a range of 1 to 10 at best. The evidence from star magnitudes covers even a smaller range. Therefore, all that can be concluded from the evidence is that within *narrow* limits the ratio $\frac{\Delta I}{I}$ may be considered constant.

This conclusion is sustained by all the work on the Weber-Fechner law subsequent to Fechner. It is not relevant to our purpose to review this material here.[1] A good deal of it has been summarized by Exner (1879), and more recently by Wundt (1908). Stripped of controversy, the evidence shows that the Weber-Fechner law holds approximately over a very moderate range of intensities. Kraepelin's (see Wundt, 1908[2]) work is an example. It was carefully done and shows a constant value of $\frac{\Delta I}{I}$ between 300 and 1,000 units of intensity, which Wundt[2] speaks of as proving Fechner's idea "*innerhalb weiter Grenzen.*" We shall see in the next section how $\frac{\Delta I}{I}$ really behaves within wide limits.

4. General Idea Involved.—It is to be regretted that in the controversies over it, the valuable kernel of the Weber-Fechner law should have failed of emphasis. Bouguer, the original discoverer, says, "Un grand bruit nous empêche d'en entendre un autre plus faible; nous

[1] It is also not in our province to discuss the various theories for the Weber-Fechner law. Most of its philosophical background (*cf.* Müller, 1903) must unfortunately be discarded because of the failure of the law to describe correctly the data of intensity recognition. From our present view-point—that of attempting to work out a mechanism of vision—a fundamental difficulty with the original law and the several modifications and elaborations proposed among others by Fechner (1860) Helmholtz (1866; 1896) and Lasareff (1914) is that, aside from their failure adequately to describe the known data, they rest on no mechanism of vision.

[2] Wundt (1908), p. 659.

ne voyons pas, en présence d'une forte lumière, une autre dont l'intensité est beaucoup moindre, si les deux frappent notre rétine dans le même endroit."[3] Weber similarly understood the matter. Sensory judgments are relative, not absolute. The attempt by Fechner to describe

TABLE I.

*Aubert's Data on Intensity Discrimination.**

I	$\frac{\Delta I}{I}$
millilamberts	
0.0005	1/3
0.0006	1/4
0.0013	1/4
0.0025	1/8
0.0056	1/11
0.0156	1/25
0.0351	1/25
0.0506	1/25
0.131	1/31
0.250	1/36
0.316	1/33
0.563	1/31
1.00	1/32
1.37	1/39
2.25	1/45
5.06	1/51
9.00	1/65
20.3	1/112
31.6	1/104
56.3	1/121
136.6	1/146

* Aubert's original figures for the intensities have been divided by 10,000 to convert them into modern units. This factor has been arrived at by comparison of Aubert's data with those of Blanchard and of Koenig and Brodhun as shown in Fig. 1.

by means of a simple formula just how relative our sensory judgments are failed unfortunately to include the full range of the phenomena.

III.

Experimental Data of Intensity Discrimination.

1. Aubert's Work.—The first experiments to determine the manner in which the ratio $\frac{\Delta I}{I}$ really varies over a wide range of intensities

[3] Bouguer (1760), p. 57.

were made by Aubert (1865), who used a procedure which is essentially the two-candles-and-a-shadow method of Bouguer. The lowest intensities were those barely perceptible; the highest were obtained from

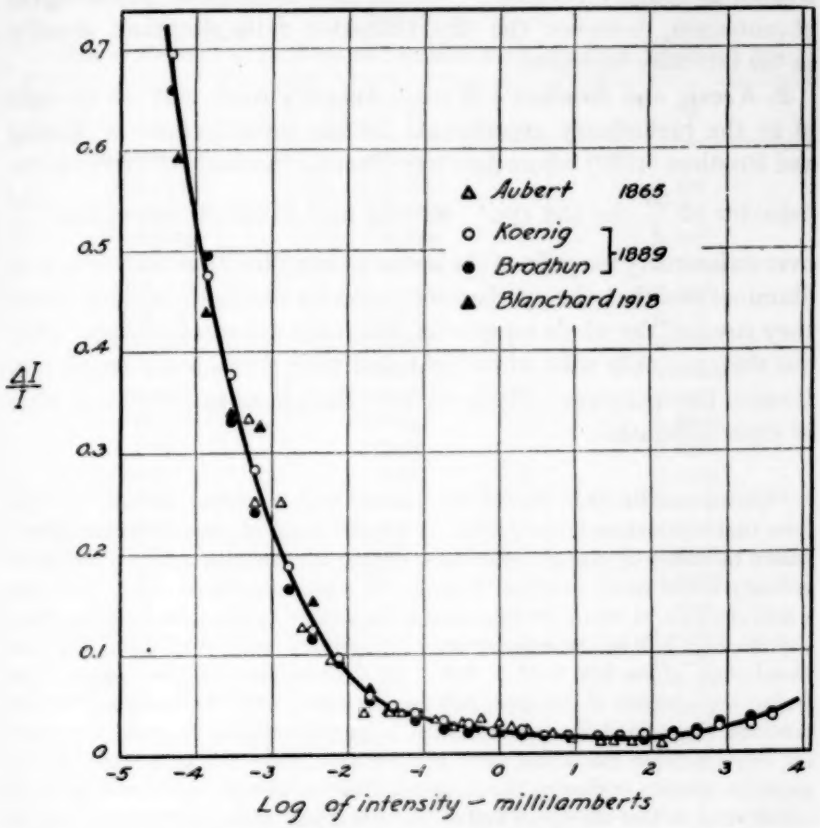

FIG. 1. Intensity discrimination over the total illumination range visible to the eye. Blanchard's data are given in their original units: millilamberts. Koenig and Brodhun's intensities have been divided by 250 in order to convert them into millilamberts. Similarly Aubert's intensities have been divided by 10,000. It is apparent that these three groups of experimenters, working many years apart and independently, have arrived at essentially the same results.

daylight admitted into the dark room. The recorded precautions involving calibration, adaptation, and the like, show a fine appreciation of the sources of error and therefore make Aubert's results reliable.

They are summarized in Table I, and graphically in Fig. 1. It is apparent that over a very limited range of illuminations $\frac{\Delta I}{I}$ may be treated as roughly constant. Viewed as a continuous physiological phenomenon, however, the discrimination ratio decreases steadily as the intensity increases.

2. Koenig and Brodhun's Work.—Aubert's work may be thought of as the preliminary experiments for the investigations of Koenig and Brodhun (1889) whose data have become the final statement of the behavior of $\frac{\Delta I}{I}$ for the eye.[4] Koenig and Brodhun determined $\frac{\Delta I}{I}$ over an intensity range from the lowest perceptible illumination to such illuminations that the eye became painfully dazzled—in other words they covered the whole range over which the eye can function. They did this, not only with white light, but with six monochromatic portions of the spectrum. The work as a whole is an extraordinary piece of experimentation.

[4] Koenig and Brodhun studied the problem with a method radically different from that of previous investigators. A parallel beam of plane polarized light is passed through a crystal of Iceland spar so that the emerging ordinary and extraordinary beams partly overlap. Viewed with a properly placed ocular this yields a field of vision, of which the upper half is illuminated by the ordinary beam alone, and the lower half by the ordinary and extraordinary beams combined. The total visual angle of the field is $6° \times 4.3°$. By the rotation of a Nicol prism in the ocular, the intensity of the upper half may be varied while the lower half remains constant, the ratio of the two intensities being proportional to $\cos^2\theta$, where θ is the angle between the ocular nicol and the axis of the calcite crystal. An observation consists in viewing the uniformly illuminated field, and then turning the ocular nicol so that the upper half of the field is just perceptibly darker than the lower. Calling the intensity of the upper half, I, the lower half will then be $I + \Delta I$, and the just perceptible difference between them, ΔI. The minimum discriminable ratio $\frac{\Delta I}{I}$ is easily computed by remembering that I is given by $\cos^2\theta$, and ΔI by $\sin^2\theta$; therefore $\frac{\Delta I}{I}$ is given by $\tan^2\theta$. The data actually secured were the intensity of the field at uniformity and the angle θ when the two halves were just perceptibly different in brightness.

Koenig and Brodhun's data for white light are reproduced in Table II and in Fig. 1.[5] The data for the different colors are essentially the same as those for white light. They may be found in the original

TABLE II.

Data of Koenig and Brodhun on Intensity Discrimination. Brodhun's Eye.

I	ΔI	$\frac{\Delta I}{I}$
millilamberts	*millilamberts*	
0.0000484	0.0000316	0.659
0.0001336	0.0000664	0.495
0.000300	0.000100	0.331
0.000644	0.000156	0.241
0.001716	0.000284	0.165
0.00358	0.000416	0.116
0.00732	0.00068	0.0912
0.0190	0.0010	0.0533
0.0382	0.0018	0.0457
0.0776	0.0024	0.0332
0.1948	0.0052	0.0270
0.3912	0.0088	0.0228
0.784	0.016	0.0223
1.956	0.044	0.0221
3.928	0.072	0.0185
7.86	0.14	0.0180
19.69	0.31	0.0158
39.36	0.64	0.0163
78.76	1.24	0.0156
196.2	3.8	0.0193
391.6	8.4	0.0215
776.8	23.2	0.0297
1,935.	65.	0.0336
3,853.	147.	0.0380

[5] The intensities given in Table II are in millilamberts, derived by multiplying Koenig and Brodhun's original figures by 0.004. They describe their unit as the brightness of a magnesium oxide screen illuminated by one tenth of a Violle standard and viewed through a 1 sq. mm. artificial pupil. The Violle standard is 23 candle power, and the reflecting power of a magnesium oxide surface is 85 per cent. The brightness of the screen is therefore 0.2 millilambert. A normal pupil at such an intensity has an effective area of about 50 sq. mm. Therefore the light reaching the eye is reduced to 1/50, which makes the unit approximately 0.004 millilambert. Blanchard's (1918) independent repetition of Koenig and Brodhun's experiments confirms the correctness of this calculation.

paper and are not reproduced here because their analysis is identical in principle with that for white light, which alone concerns us in the present paper.

3. Corroborative Evidence.—These data support those of Aubert in showing that the ratio $\frac{\Delta I}{I}$ not only is not constant as demanded by the Weber-Fechner law, but that it varies in a definite way. Koenig and Brodhun's work covers intensities much higher than those of Aubert, and at these, the ratio, after first decreasing now begins to increase.

This rise at high intensities is undoubtedly a real phenomenon, first because of the intrinsic excellence of the experiments themselves, and second because the same increase had been found for intensity discrimination in *Mya* (Hecht, 1923–24, *a*) by a method entirely different. That the other parts of the data are also not open to question has been shown by Blanchard (1918) who repeated the experiments in still another way. Blanchard gives $\frac{\Delta I}{1+\Delta I}$ but from his published data it has been simple to compute $\frac{\Delta I}{I}$. These values are plotted in Fig. 1. The points in Fig. 1 show clearly that the three groups of independent experiments covering half a century have yielded identical results.

We possess, therefore, a reliable set of figures, free from preconception, which record accurately the discrimination threshold at all intensities at which the eye can function. In the further treatment of these data, we shall confine ourselves to those for Brodhun's eye alone. The data for Koenig's eye are practically the same, and their theoretical analysis is identical.

IV.

Influence of Pupillary Size.

1. Principle of Analysis.—The experiments of Koenig and Brodhun measure the discriminating power of the eye as a whole. The data are thus a synthesis of the effect of light on at least three structures in the eye: the iris, the rods, and the cones. It is our object to describe these data in terms of a mechanism of vision. There-

fore, it becomes necessary to isolate the individual contribution of each of these three systems toward their combined effect. In this way it will be possible to analyze the actual relations between light and the photosensory process in the retina.

Intensity discrimination is obviously determined by the amount of light which enters the eye and falls on the rods and cones. However, the data as they stand record the outside intensities and do not give the real sequence of illuminations at the retina, because between the light outside and the retina inside there is a variable diaphragm—the iris—whose opening itself varies with the intensity. The pupil is therefore the first system whose contribution must be isolated and eliminated.

TABLE III.

Relation between Intensity and Pupil Area during Monocular Illumination as Computed from Reeves' Data.

I	Area.
millilamberts	*sq. mm.*
0.0	50.9
0.00015	48.4
0.01	48.4
0.6	40.2
6.3	26.4
126.0	8.55
355.	6.60
2,000.	3.14

2. Elimination of Pupil as Factor.—The removal of the iris as a factor is accomplished by means of the work of Reeves (1918). Koenig and Brodhun's experiments involve looking with one eye through a telescope. The other eye, whether open or shut, is in the dark. Table I of Reeves' paper records the relation between intensity and pupil width under monocular illumination. From these figures the area of the average pupil at different illuminations has been calculated; the results are given in Table III and graphically in Fig. 2.

With any value of the intensity for Brodhun's eye one can find by graphic interpolation the corresponding pupil area. These areas are in Column 3 of Table IV. The product of the outside

intensity and the resulting pupil area gives the figures in Columns 4 and 5 of Table IV. These then represent the actual sequence of illumination intensities at the retina corresponding to the series of discrimination ratios in Table II.

Having in this way removed the iris as a variable in these data, we must now consider their meaning in terms of the effect of light on the rods and cones.

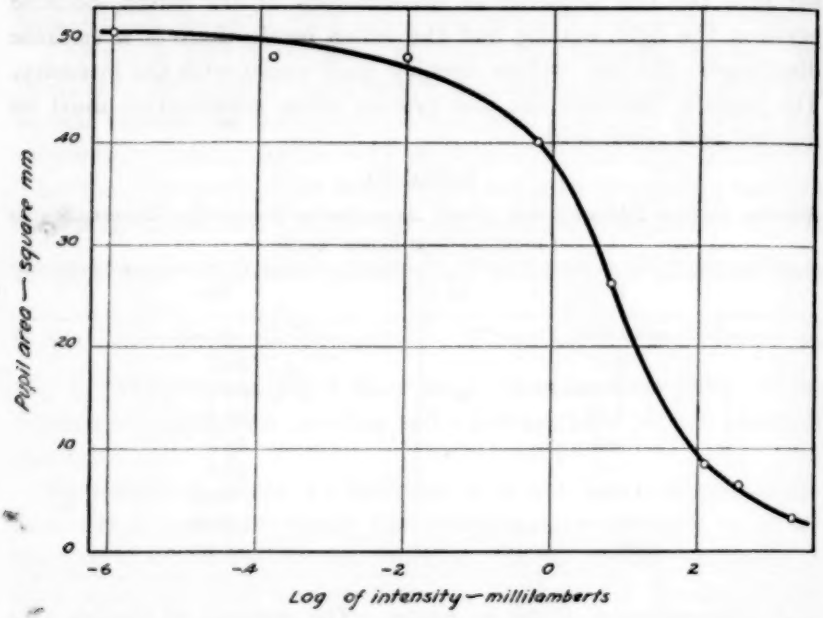

FIG. 2. Relation between intensity and pupillary area with monocular illumination. The points are computed from Reeves' data, and are the averages of two observers.

V.

Retinal Basis of Intensity Discrimination.

1. Separation of Rods and Cones.—The rods and cones of the retina are two separate photosensitive systems, with different thresholds, different rates of dark adaptation, and in general different functions. They possess in common, however, the capacity for intensity discrimination, because intensity differences can be observed with the

fovea alone, which contains only cones, as well as at such low illuminations that only the rods function. It may be possible to identify the separate contribution of each of these two systems in intensity discrimination with the aid of their differences in other respects.

The visual field in the experiments of Koenig and Brodhun includes both retinal elements. At the lowest perceptible intensities the rods alone mediate intensity discrimination. As the intensity increases a point is reached which lies at the cone threshold. From then on, either the value of ΔI is determined as a sort of average between the rods and cones, or at some point the cones take over completely the function of intensity discrimination, provided the necessary increase ΔI is less for the cones than for the rods. The first alternative, though possible, is complicated, and does not help us to separate the action of the two types of sense cells. The second alternative—a sharp division—is simpler, and more useful because its consequences are clear. As the intensity increases, analysis must show a break at some point, preceding which ΔI has been determined by the rods, and beyond which it is controlled by the cones.

The separation of the two retinal elements in this way is a cardinal feature of the present treatment of the data. It leaves us free to consider the rods and cones as separate systems and to study the action of light on each independently.

In order to do this effectively, we shall find it convenient to examine intensity discrimination in *Mya*, where its nature and mechanism have already been described (Hecht, 1923–24, *a*). The significance of *Mya* for the study of the visual process lies in the fact that its photosensitive system is a simplified version of the eye. It possesses neither lens nor iris, and apparently only one kind of photosensitive cell. Its outstanding qualification is that the chemical mechanism underlying its photic sensitivity corresponds in many essentials to that of the rods and cones. The properties of the mechanism in *Mya* have been studied in a variety of ways, and in the present instance of intensity discrimination the behavior of the mechanism will serve as the basis for the analysis of the similar discrimination by the retina.

2. Intensity Discrimination in Mya.—Mya, when first exposed to a given illumination, responds by the retraction of its siphon. Continued exposure produces no further reponse, and the animal, after extending its siphon again, comes into sensory equilibrium. If the intensity of the illumination is raised by a certain increment, another response occurs. *Mya* can therefore distinguish between two intensities to which it is successively exposed, and indicates this discrimination objectively by the retraction of its siphon. When studied over a large range of intensities the quantitative results show an extraordinary similarity to the data for the eye. As the intensity rises, the ratio $\frac{\Delta I}{I}$ gradually decreases to a certain point, after which it increases again. It fact, the curve for the relation between $\frac{\Delta I}{I}$ and log I (Fig. 1) for the eye and for *Mya* are almost identical (*cf.* Figs. 4 and 5 in Hecht, 1923–24, *a*).

The analysis of the results on *Mya* is secured in terms of its underlying photochemical mechanism. This system may be represented for mathematical convenience as a reversible reaction of the type

$$S \underset{\text{"dark"}}{\overset{\text{light}}{\rightleftharpoons}} P + A \qquad (3)$$

whose properties have been derived from the responses of *Mya* under conditions designed for that purpose. A characteristic of this reaction system is its capacity to come into an apparently stationary state under continued illumination. This results in the light adaptation of the animal. When illuminated, the sensitive material S absorbs light and is changed into P and A, the velocity of the reaction depending on the concentration of S and on the intensity of the light. The reverse reaction, being independent of light, sets in as soon as some P and A are formed, the velocity of recombination being proportional to the concentrations of P and A. If the illumination is maintained, the two opposing reactions quickly strike a stationary condition in which the concentrations of S, P, and A remain constant. This point is determined by the intensity of the illumination, and may be described in terms of a simple equation.

The data on *Mya* show that when the animal discriminates between one intensity and another, the transition from the stationary state of the reaction $S \rightleftharpoons P + A$ at one intensity to the stationary state at the other is accompanied by the decomposition of a definite quantity of photosensitive substance. Thus, discrimination between two intensities involves a constant amount of photochemical decomposition, regardless of the numerical values of I and ΔI and their ratio.[6]

3. Outline of Analysis in Eye.—Studies of the dark adaptation of the eye (Hecht, 1919–20; 1921–22) have shown that a reversible photochemical reaction similar to that in *Mya* exists in the rods and in the cones. Our treatment of intensity discrimination in the eye may then be expressed in terms of its two photochemical systems with reference to their resemblances to that of *Mya*. At low intensities the rods alone determine discrimination. The photochemical system comes to a stationary state at a given value of I. Then, in order for the rods to recognize a further increase in intensity the additional light ΔI must be of such magnitude as to decompose a given amount of photosensitive material. This results in a constant difference in the concentration of sensitive material between the stationary state determined by I and that determined by $I + \Delta I$. For the rods the absolute magnitude of this constant increment in decomposition is larger than it is for the cones. Therefore the recognition of intensity differences shifts to the cones at an illumination where ΔI furnishes sufficient energy to produce in them the necessary decomposition but not enough to produce the corresponding change in the rods. From then on the cones continue to determine intensity discrimination until the concentration of S at the initial stationary states is so low that ΔI, no matter how great, cannot decompose the necessary amount of sensitive material.

It is apparent from even this general statement of the retinal basis of intensity discrimination that a mechanism as simple as this

[6] The idea that a constant increment in photochemical decomposition is the condition for a threshold difference in sensation has already been put forward by Cobb (1916). In addition Cobb conceived the notion of a reversible reaction as the basis for a visual mechanism, and even derived an equation for its stationary state.

cannot be complete. The phenomena involved are diverse and complex. The best that can be done at present is to use these ideas as the basis for a first approximation, simple enough for rigorous, quantitative consideration but broad enough for the necessary future modifications. The mechanism here proposed obviously involves a number of aspects of vision, the exact properties of which will have to be in harmony with the results of a quantitative treatment of the data. We shall return to this phase of the matter after first subjecting the data of Koeing and Brodhun to such an analysis.

VI.

Quantitative Analysis of Intensity Discrimination.

1. Calculations and Results.—The photochemical system in the rods and in the cones both may be represented for convenience as reversible reactions of the type

$$S \underset{\text{"dark"}}{\overset{\text{light}}{\rightleftharpoons}} P + A. \tag{4}$$

Let such a system be exposed to light of intensity I, and let the illumination be maintained until x per cent of P and A has been formed, leaving $(a - x)$ per cent of S, the original concentration of which was a. At that moment the velocity of the light reaction alone, $S \rightarrow P + A$, since it is proportional to the concentration and to the intensity (Hecht, 1923–24, *b*), is given by

$$v_1 = k_1 I (a - x). \tag{5}$$

Because the complete reaction is reversible, P and A reunite to form S independently of light. The velocity of their recombination is proportional to the concentrations of P and A, and is given by

$$v_2 = k_2 x^2. \tag{6}$$

As the illumination is continued the two velocities become equal, and the resulting stationary state is described by

$$K I = \frac{x^2}{a - x} \tag{7}$$

in which $K = \frac{k_1}{k_2}$. This equation[7] of the stationary state is all that is necessary for the computation of the data in terms of our analysis. It contains only two variables—I, the intensity, and x, the concentration; of these, I is clearly the independent variable, and its value at once determines the equation.

In the retina let x_1 be the concentration of P and A at the stationary state of the intensity I, and let x_2 be the corresponding concentration for $I + \Delta I$. Then a value of the constant K can be found for which the quantity $x_2 - x_1$, computed from the data, is constant for a series of intensities beginning with the lowest. As the intensity increases the value of $x_2 - x_1$ should fail to conform at a certain point, which represents the illumination where the rods cease to determine intensity discrimination. Beginning with this intensity a new value of the constant K should be found which will again render $x_2 - x_1$ constant but of smaller magnitude than before.

Computation of the data yields a set of figures which support such a line of reasoning. Table IV contains the intensities I and $I + \Delta I$ at the retina, the values of x_1 and x_2 of the two corresponding stationary states, and the two values of K. Taking $K = 100$, it is plain that the first seven intensities yield practically constant values of $x_2 - x_1$. The average value for the seven is 1.56, which repre-

[7] Equation (7) describes the stationary state in the simple terms of a completely reversible reaction. Very likely the system is really more complicated, and is probably only pseudoreversible, such as

$$\begin{matrix} & \overset{\text{light}}{\longrightarrow} & \\ S & & P + A + B \\ S & \underset{\text{"dark"}}{\longleftarrow} & P + A + C \end{matrix} \qquad (8)$$

in which B is different from C, and C is present in excess. The mathematical treatment for the two, however, is the same. In case of the pseudoreversible reaction the stationary state represents not only a constant concentration of S, P, and A, but in addition a steady production of B at a rate proportional to the concentration of P and A. Probably it is this continuous formation of B, and its effect on the nerve which enable us to see an illumination to which the eye is adapted. It is possible that a similar pseudoreversible reaction underlies the behavior of many animals which continue to orient to light after they have become adapted to it.

sents the unitary change for rod vision. For the next few intensities, if calculated with $K = 100$, the quantity $x_2 - x_1$ drops sharply first to 0.90 and rapidly almost to 0. If now the value of K be put at 0.25 a new series of values of $x_2 - x_1$ is secured, smaller in magnitude and roughly constant at an average value of 0.21. In terms of the analysis this is the unitary change for the cones.

TABLE IV.

Concentrations of Sensitive Substance Decomposed at Different Outside Illuminations.

Illumination outside.		Pupil area.	Illumination at retina.		Concentration.			K
I	$I+\Delta I$		I	$I+\Delta I$	x_1	x_2	x_2-x_1	
0.0000484	0.0000800	50.3	0.00243	0.00402	4.81	6.14	1.33	100
0.0001336	0.000200	49.7	0.00664	0.00994	7.83	9.48	1.65	
0.000300	0.000400	49.5	0.0149	0.0198	11.48	13.12	1.64	
0.000644	0.000800	49.0	0.0316	0.0392	16.27	17.94	1.67	
0.001716	0.00200	48.6	0.0834	0.0972	25.01	26.69	1.68	
0.00358	0.00400	48.0	0.172	0.192	33.76	35.26	1.50	
0.00732	0.00800	47.7	0.349	0.382	44.15	45.59	1.44	
0.0190	0.0200	47.4	0.901	0.948	4.63	4.75	0.12	0.25
0.0382	0.0400	46.9	1.79	1.88	6.47	6.63	0.16	
0.0776	0.0800	46.3	3.59	3.70	9.04	9.17	0.13	
0.1948	0.2000	44.3	8.63	8.86	13.65	13.81	0.16	
0.3912	0.4000	42.0	16.43	16.80	18.32	18.50	0.18	
0.784	0.800	38.6	30.26	30.88	23.75	23.95	0.20	
1.956	2.000	33.6	65.72	67.20	33.14	33.45	0.31	
3.928	4.000	29.2	114.7	116.8	41.12	41.37	0.25	
7.86	8.00	24.4	191.8	195.2	49.30	49.59	0.29	
19.69	20.00	18.3	360.3	366.0	60.02	60.28	0.26	
39.36	40.00	14.2	558.9	568.0	67.45	67.71	0.26	
78.76	80.00	10.7	842.7	856.0	74.00	74.24	0.24	
196.2	200.0	7.4	1,451.9	1,480.0	81.64	81.88	0.24	
391.6	400.0	5.7	2,232.1	2,280.0	86.57	86.79	0.22	
776.8	800.0	4.5	3,495.6	3,600.0	90.61	90.83	0.22	
1,935.0	2,000.0	3.2	6,192.0	6,400.0	94.26	94.43	0.17	
3,853.0	4,000.0	2.2	8,476.6	8,800.0	95.68	95.83	0.15	

The values of $K = 100$ for the rods and $K = 0.25$ for the cones were found by trial, and are obviously round numbers. They show clearly, first that $x_2 - x_1$ is smaller for the cones than for the rods, second that there is a sharp break at a certain illumination when intensity discrimination shifts from the rods to the cones, and third that $x_2 - x_1$ for the rods and the cones has in each case a constant

order of magnitude which represents the unitary minimal decomposition of sensitive substance necessary for intensity discrimination.

2. Sources of Error.—The values of $x_2 - x_1$ for the rods though perhaps varying in a regular way, on the whole are reasonably constant. The values of $x_2 - x_1$ for the cones—all within another order of magnitude—do, however, vary definitely in a regular manner. In the computations the values of x are obtained as differences between two larger numbers. Too much reliance, therefore, is not to be placed on the precise numerical values of $x_2 - x_1$ for the cones, because they are second order differences, and lie at the limit of accuracy of the data. They show a reasonable similarity and a constant order of magnitude, and satisfy the requirements of an analysis which is a first approximation.

There are several aspects of the experiments which would influence the data and hence the constancy of $x_2 - x_1$. In the first place the values of Koenig and Brodhun were corrected by means of the data of Reeves on pupil size. Koenig and Brodhun looked through a telescope at a small illuminated area surrounded by darkness, whereas Reeves used a large illuminated surface. The two are therefore not strictly comparable measurements (Cobb, 1916). In the second place it is possible that the ocular opening of the telescope used by Koenig and Brodhun was smaller than the pupil at maximum dilatation. This would influence the measurements at low intensities. These two sources of error are probably small in magnitude and their existence may even be doubtful.

A more serious matter concerns the relation of the two halves of the field as projected on the retina. Our analysis tacitly assumes that when two contiguous parts of the retina are illuminated one by I and the other by $I+\Delta I$ the same results obtain as when the same area is subjected successively to these just discriminable intensities. Cobb (1916) has shown that the part of the retina which surrounds an illuminated area plays a distinct rôle in intensity discrimination. A similar effect is to be deduced from the work of Lasareff (1911) on the influence of the size of the field on intensity discrimination. Very likely two differently illuminated contiguous areas in the retina affect each other, and this influence would distinctly depend on the intensity, and hence would be different for different parts of the data.

Still another influence in the constancy of $x_2 - x_1$ concerns the

method of deriving the equation for the stationary state. In writing the velocity of the light reaction alone as directly proportional to the incident intensity it has been assumed (Hecht, 1923–24, *b*) that the absorption coefficient of the sensitive substance is small. This, though accurate for first order results, is probably inaccurate for second order computations such as those represented by $x_2 - x_1$ for the cones. For the sake of simplicity the assumption is retained, but it must be borne in mind as a source of error.

3. Trimolecular Assumption and Results.—Nevertheless there is a slight modification in the detail, but not in the principle, of the analysis which eliminates at once the regular variation in $x_2 - x_1$ for the cones. The bimolecular nature of the "dark" regeneration reaction rests on the kinetic interpretation of dark adaptation data (Hecht, 1921–22) and represents the simplest reaction order compatible with the observations. Within the experimental error there is no great difference between the kinetics of a bimolecular reaction and a trimolecular one. If we assume the dark reaction to be trimolecular the equation for the stationary state becomes

$$K I = \frac{x^3}{a - x}. \tag{9}$$

The values of $x_2 - x_1$ for the cones derived from this equation show an excellent degree of constancy.

Using $K = 20$ for the cones, the following values of $x_2 - x_1$ are obtained[8] corresponding to every other set of cone intensities in

[8] Since the direct computation of x from equation (9) is not possible, a graphic method has to be used. Giving for the moment a value of 1 to K, and assuming a series of values for x between 0 and 100 per cent, the resulting values of I are calculated. A large scale curve relating x and $\log I$ is then plotted. The curve has an S shape similar to those in Fig. 3. The distance on this curve between any pair of values of $\log I$ and $\log (I + \Delta I)$ is so small that it may be considered with great accuracy as part of a straight line. From this the value of x can be found as

$$x = a \log I + b \tag{10}$$

in which a is the tangent of the curve at the point under consideration. The difference between x_2 and x_1 is then

$$x_1 - x_2 = a \log (I + \Delta I) - \log I. \tag{11}$$

The first differential of the large scale curve is constructed giving the relation between $\log I$ and the tangent a, after which it is simple to choose a proper value for K and to compute $x_2 - x_1$ for the values of I and $I + \Delta I$.

Table IV: 0.20, 0.17, 0.19, 0.23, 0.21, 0.22, 0.23, 0.20, 0.19. These values are obviously constant. Their average is 0.20 representing the unitary difference in stationary state between x_1 and x_2, and is probably identical with the average of 0.21 found by use of the bimolecular assumption.

The reasons that the bimolecular assumption is used in presenting the data are, first, that it is simpler to handle, and was the one found for dark adaptation; second, as far as the general analysis is concerned, the differences in the two results are really matters of detail and not very significant; third, the trimolecular assumption breaks down with the rod data: it has not been possible to secure constant values of $x_2 - x_1$ for the rods by the use of equation (9). The choice is therefore between an approximately good fit for both rods and cones with a bimolecular assumption, or a good fit for the rods with the bimolecular and for the cones with the trimolecular. We have adopted the first. If the second alternative has to be chosen later nothing is lost, and much is gained in an understanding of the situation. The theoretical consequences are practically the same for the two.

The main point to be brought out is that a quantitative treatment of the data of intensity discrimination yields results which fit with a reasonable degree of accuracy the significant features of the mechanism outlined for the photochemical basis of intensity discrimination.

VII.

Facts Related to Present Analysis.

1. Threshold of Rods and Cones.—There are a number of facts of vision which can be applied as tests for the consistency of the present treatment of intensity discrimination. We have assumed—and the analysis of the data has borne it out—that the differential threshold ΔI is smaller for the cones than for the rods. How does this agree with the fact that the cones are known to have a higher *absolute* threshold than the rods? Fortunately there is not only no contradiction between these two facts, but the analysis here proposed actually calls for the higher absolute threshold.

The simplest way of showing this is by plotting equation (7) of the stationary state using x as ordinates and log I as abscissæ. Put-

ting $K = 100$ gives the left hand curve in Fig. 3, and putting $K = 0.25$ gives the right hand curve. The curves are parallel and represent the behavior of the photochemical system in the rods and cones respectively. The points are the computed values of x_1 of Column 6 corresponding to I in Column 4 of Table IV.

The values of K used in the calculations in this paper were chosen with no other desire than to get the most constant values for $x_2 - x_1$

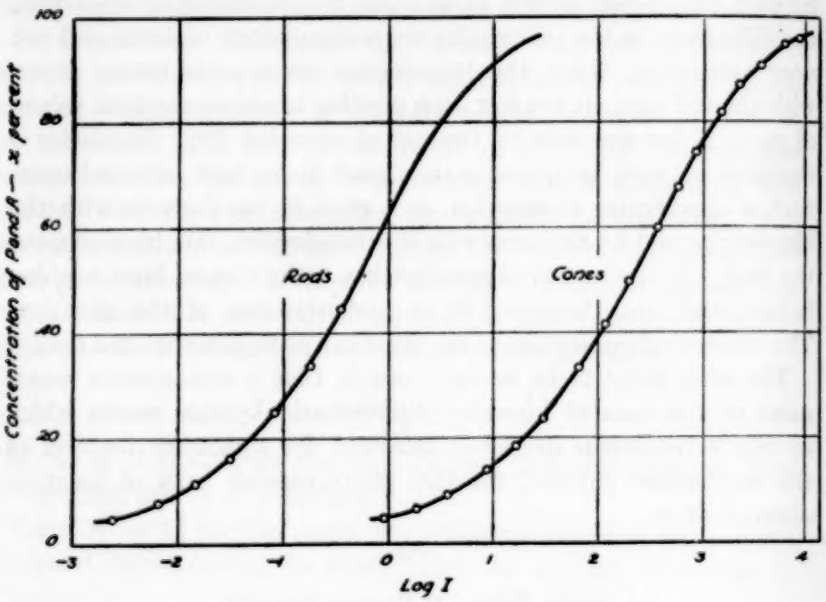

FIG. 3. Relation between retinal illumination and the amount of photochemical action at the stationary state of the reaction $S \rightleftharpoons P + A$. The curves represent the equation $K I = \frac{x^2}{a-x}$, in which $K = 100$ for the rods, and $K = 0.25$ for the cones.

for the two systems. It is significant that the curves which result from the use of such values of K indicate a higher absolute threshold for the cones than for the rods. The whole cone system seems to be pitched at a higher intensity than the rods. The analysis of the data in terms of the suggested mechanism is therefore in harmony with this familiar truism of retinal physiology.

2. Dark Adaptation.—The relative values of K for the rods and cones have an interesting bearing not only on the absolute thresholds of the two systems, but on their dark adaptation as well. It will be remembered that $K = \frac{k^1}{k^2}$ in other words that K is the ratio of the velocity constants of the light and "dark" reactions in the photochemical system. For the rods $k_2 = 0.01\ k_1$, whereas for the cones $k_2 = 4\ k_1$. These do not tell us the absolute values of k_2 for the rods and cones because k_1 is probably different for the two. But they create a strong presumption that k_2 for the cones is larger than for the rods. The rate of dark adaptation is a function of the "dark" reaction whose speed is determined among other things by the magnitude of k_2. From this it follows that the cones should have a more rapid rate of dark adaptation than the rods. The data on this point are unequivocal. The dark adaptation of the cones is practically complete in 3 minutes (Hecht, 1921–22) whereas the same process in the rods is not complete in 30 minutes (Hecht, 1919–20).

An additional point of interest in regard to dark adaptation is the relation between intensity and photochemical effect. It is apparent from Fig. 3 that between 10 and 90 per cent both curves may without great distortion be treated as straight lines. Therefore, within a reasonable experimental error the effect of light may be considered as proportional to the logarithm of its intensity. This holds true also when the dark reaction is trimolecular. It is to be noted that in order to interpret the data of dark adaptation of the rods in terms of a reversible photochemical reaction this is precisely the relation which we assumed between intensity and photochemical effect.

For the cones the simplest assumption to fit the data (Hecht, 1921–22) is that the effect is directly proportional to the intensity. Recomputation of the data in terms of the log I relation, however, also gives a bimolecular order to the dark reaction.[9] The linear

[9] The reason that both assumptions yield similar results lies in the fact that the range of threshold intensities, and therefore of the photochemical effects for cone dark adaptation are comparatively small. Combining equations (5) and (6) for the light and dark reactions respectively the velocity of the process as a whole can be expressed as

$$v = k_1 I (a - x) - k_2 x^2. \qquad (12)$$

If the amount of photochemical change x is small, say less than 10 per cent, x^2

relation and the logarithmic relation are both approximations, the latter being more nearly correct, since it covers a much larger range. However, the only strictly exact relation between intensity and photochemical effect is equation (7) for the stationary state on the one hand, and on the other the integral of equation (12) for the course of the reaction. This integration has been carried out and tested experimentally (Hecht, 1922–23).

Free from any assumptions, however, the experimental data of dark adaptation show clearly that the cone process is faster than the rod process. And this is the main point relevant to our present analysis.

3. Transition Point in Visual Acuity.—Perhaps the most striking outcome of our analysis is the presence of a sharp change in the magnitude of $x_2 - x_1$ representing the transition from rods to cones. The data themselves present no such break (*cf.* Fig. 1). The presence according to our analysis of such a clean break in the calculations, therefore, requires substantiation from some other source in order to render its reality more probable. Fortunately there exists a set of data which furnishes precisely this corroboration.

Koenig (1897) investigated the relation between visual acuity and intensity of illumination. The range of intensities in the experiments covers the interval from the lowest utilizable intensities to those which result in no further increase of visual acuity. The results show that visual acuity is proportional directly to the logarithm of the intensity. For white light, beginning with the lowest intensity ($I = 0.00036$ unit) the data, plotted as acuity against $\log I$, fall on a straight line whose tangent is 0.0414. At very nearly 0.1 unit of intensity, the data sharply diverge, and from now on fall also on a straight line whose tangent, however, is 0.434 or about ten times that for the low intensities.

For intensities below the intersection of the two lines Koenig found that visual acuity is controlled by the periphery of the eye,

becomes less than 1 per cent, and the minus term may be neglected. At the same time $(a - x)$, being ever 90 per cent, may be considered constant. This yields that the velocity of the reaction, and therefore the amount of decomposition for comparable exposures is directly proportional to the intensity.

fixation with the fovea being impossible; but above the intersection the cones come into play and fixation is entirely foveal. These observations were confirmed beautifully by Koenig in a similar investigation of visual acuity in a totally color blind person. In this case the relation between visual acuity and log I is given by a *single* straight line identical in slope and position with the lower straight line for Koenig's own eye.

From our standpoint the implications of all this are clear, because visual acuity is an indirect manifestation of intensity discrimination. Up to a certain point in the intensity scale, acuity is determined by the rods; above that point it is determined by the cones. The point of transition should correspond in some way with the one determined in our analysis of intensity discrimination. The transition intensity is 0.1 units, and is therefore 278 times the threshold intensity. The threshold for intensity discrimination is 0.0000484 millilambert, (Table IV), and 278 times this threshold gives 0.0134 millilambert, a value midway between the last intensity for the rods (0.00732 millilambert) and the first intensity for the cones (0.0190 millilambert).

The transition point from rods to cones as derived from acuity and discrimination data is obviously the same *relative* to the threshold of visibility in the two cases. It would be added evidence to determine whether the *absolute* values of the transition intensity in the two cases are of the same order of magnitude. The units in the data for intensity discrimination have been defined. It is necessary only to find the unit of intensity for the acuity data. Koenig defines this as the illumination by a Hefner lamp at a meter distance. This is 0.9 meter candle; and if the surface reflects 100 per cent, corresponds to 0.09 millilambert. Obviously white paper reflects less than that; how much less we cannot tell from the information furnished. Assume 80 per cent reflection[10] since the paper was evidently chosen with care. This gives the unit a value of 0.072 millilambert. The transition point at 0.1 unit then becomes 0.0072 millilambert, which (Table IV) is exactly the value of the last intensity controlled

[10] Sumpner (1893) found that white blotting paper reflects 82 per cent; white cartridge paper 80 per cent; and ordinary foolscap 70 per cent of the incident light.

by the rods. A slight difference is to be expected between the two groups of results in view of Cobb's work on the influence of the background on intensity discrimination. The acuity work was done with a field of vision most of which was uniformly illuminated, whereas intensity discrimination was measured with a small illuminated area against a background of physical blackness. Nevertheless the agreement is surprisingly close.

An additional point of significance in Koenig's acuity measurements is the position of the transition point in relation to the total range over which the rods can function. From Table IV and Fig. 3 it is apparent that the transition point corresponds to a condition in which approximately half the sensitive substance of the rods has been decomposed, and therefore lies about midway between the limits over which the rods can function. The straight line which describes the acuity data of the color blind individual in Koenig's experiments, and consequently corresponds to the activity of the rods, is almost accurately bisected at the transition point in the data of Koenig's own eye.

Such a series of correspondences between acuity measurements and the calculations from the intensity discrimination data in terms of our analysis can hardly be mere coincidences. When there is added the evidence of the relative thresholds and the agreement with adaptation data, it seems fair to conclude that the assumptions on which our analysis is based as well as the results calculated in terms of it from the data of Koenig and Brodhun probably represent something real in the composition and action of the rods and cones in the visual discrimination of intensity.

VIII.

Discontinuity and the Structural Basis for Intensity Recognition.

1. Discontinuity of Intensity Recognition.—Historically, the idea behind the Weber-Fechner law was an effort to describe the nature of intensity recognition in terms of intensity discrimination. There are two reasons for the failure of the law to do this. First, as must be abundantly clear from our present paper, it was based on data which do not describe intensity discrimination correctly. Second,

it omitted recognition of the obvious discontinuity of intensity perception. This is particularly true of the integrated form—the psychophysical law of Fechner—since the very act of integration assumes infinitesimal changes rather than crude discontinuities.

However, the fact that visual recognition of intensity proceeds in a series of discrete steps is of fundamental importance and must form the basis of any acceptable analysis of the phenomena. Koenig (1895) calculated from the data of Koenig and Brodhun that 572 such steps cover the perception of the entire range of intensities. Our own calculations (Table IV and Fig. 3) indicate that about one-third of these steps are discriminated by the rods, the rest by the cones.

2. Basis of Discontinuity in Retina.—In terms of our analysis, each of these perceptible steps in intensity recognition represents a unitary increase in the quantity of sensitive material decomposed in the retina. It is important to consider the exact nature of these unitary increases. We have pictured them as occurring in the retina. The experiments of Müller (1897) and more recently of Brückner and Kirsch (1913) and of Lasareff (1923, *b*) have made it certain that even extreme changes in the light and dark adaptation of the eye do not influence its threshold for electrical and mechanical stimulation. These, therefore, represent the threshold of the structures which lie central to the rods and cones, since during adaptation the peripheral sense cells undergo enormous changes in sensitivity to light. Intensity differences on the retina result in local differences in adaptation. Since these cause no changes in the sensitivity of such structures as the ganglion cells and the optic centers in general, it follows that changes in sensitivity to light which do exist must be caused by the changes in the retina alone.

Aside from the direct bearing that this conclusion has on the matter in hand, it is also interesting as demonstrating (*cf.* Hecht, 1922–23) that a sense organ like the eye acts as a sort of buffer between the environment and the central nervous system.

3. Entire Process in Each Retinal Element.—One way of conceiving the nature of these unitary increments in photochemical decomposition in the retina is to consider each rod and cone as a complete system capable by itself of covering the range of intensity perceptions

and discriminations indicated by the two curves in Fig. 3. Transmission to the respective nerve fiber of such a series of unitary increases may be pictured in a speculative way as follows: Each intensity is represented by a discontinuous, all-or-nothing discharge from the sense cell, the rhythm of which is determined in part by the concentration of decomposed material, or more likely by the rate of energy liberation in the process of decomposition.[11] A unitary increase in this rhythmic discharge means a minimal increase in intensity, and the frequency corresponding to any stationary state would determine intensity perception as a whole. This is essentially the idea proposed by Forbes and Gregg (1915–16) to account for intensity transmission in reflexes, but modified to correspond with unitary increments (*cf.* also Adams and Cobb, 1922).

4. Each Element Represents One Step.—Such a conception of intensity recognition would mean that in a given illuminated retinal area all the rods and all the cones, even at their thresholds, function at the same time. This accounts not only for the present data, but also for those of dark adaptation. It must be apparent nevertheless, that this is probably one of those simplifications of the problem to which reference was made at the outset of our analysis. On such a basis it would be difficult to understand so patent a fact as the increase of acuity with intensity. It may be that more cones and more rods are functional at the higher intensities than at the lower.

This reasoning can be carried to its logical end by assuming that each sensory element behaves strictly in an all-or-nothing manner and that unitary increments in the retina represent the addition of a group of sense cells to those already functioning. In this case intensity recognition would be determined solely by the number of sensory elements acting in a given area. Such an idea, following from the development of the all-or-nothing hypothesis in nerve (Adrian, 1913–14) has been suggested frequently before (*e.g.* Forbes and Gregg, 1915–16), but more recently again by Lasareff (1923, *a*),

[11] Such a scheme is particularly consistent with a pseudoreversible reaction like the one suggested in Section VI. At the stationary state, light is being steadily poured in at one end of the sense cell. The result is a continuous production of B, which heaps up and discharges rhythmically, and stimulates the nerve ending at the other end of the cell.

who derives it from the anatomical discontinuity of the retina and the quantal discontinuity of light. This kind of hypothesis is very attractive, though unfortunately its quantitative implications have never been described concretely with reference to any series of data.

In considering the quantitative consequences of such an interpretation it clearly is insufficient to rely, as Lasareff has done, merely on the all-or-nothing character of the nerve impulse plus the discontinuity of both retina and light. There must be made the additional and more critical assumption that the sensory elements in the retina differ widely in their threshold. It is on the basis of this quantitative variation in sensory threshold that such an idea can be used to describe the data of intensity discrimination.

It is simple to show that because of this variability in the sensory elements this type of arrangement can also be based on the photochemical mechanism derived in this paper to account for intensity discrimination. Assume that when they differ in threshold at all, the sense cells differ by the unitary amount $x_2 - x_1$ in the quantity of decomposed photosensitive material necessary to set off an impulse from the cell. If there are the same number of sense cells corresponding to each unitary step in concentration, their distribution *with relation to the intensity* will be such as to give the two curves in Fig. 3. The resemblance of these two curves to integral distribution curves is striking. The form of their first differential is distinctly that of the usual frequency curves of populations, errors, and the like. It is not symmetrical, as is apparent from the point of inflection which lies not at 50, but near 60 per cent. This is a consequence of the fact that equation (7) which determines the curves is itself unsymmetrical. In the case of the excellent fit for the cones given by equation (9) the first differential curve is still more skew, since the equation is obviously still less symmetrical. It is therefore clear that these consequences of our analysis can easily serve as the ultimate basis for an interpretation of the variability of the sense cells. The present photochemical analysis may thus be considered basic.

5. Combination of Two Extreme Ideas.—The obvious notion that each cone and each rod can cover the whole range of intensity discrimination is probably too simple to be the correct expression of our analysis. The opposite idea that each cone and each rod can cover

only one step in the series of intensity discriminations seems an extreme form of functional limitation, for which there is no proof. Moreover, it fails to explain the phenomena of dark and light adaptation.

It is possible of course to consider a compromise between these two plans. In this each cone and each rod would contain a complete photochemical system capable of discriminating intensities over a small range, say five or ten unitary steps, and in addition different individuals would be pitched at higher intensity levels somewhat as the cones as a whole are higher than the rods. The levels between the individual cells would be less than the range that can be covered by a single cone or rod. In this way a step in intensity discrimination would mean a unitary step in a given sense cell or the addition of a new one to those already affected; and in an area under illumination probably both of these would occur at once. The curves in Fig. 3 would then represent the smoothed result of the combined effects. This compromise mechanism explains not only such things as the dependence of acuity on intensity and the variation of the discrimination ratio with the size of the visual field but also the curious interchangeability of intensity and the size of the visual area (*cf.* Parsons, 1915[12]). Such an idea receives additional support from the fact that several rods may be supplied by a single nerve fiber.

At present there seems to be no means of choosing among these structural interpretations of our analysis. Experiments must therefore be devised whose purpose will be to throw light on these fundamental aspects of vision. Such experiments have already been planned, and it is hoped that the results and their interpretation will be forthcoming in the near future.

SUMMARY.

1. A study of the historical development of the Weber-Fechner law shows that it fails to describe intensity perception; first, because it is based on observations which do not record intensity discrimination accurately, and second, because it omits the essentially discontinuous nature of the recognition of intensity differences.

[12] Parsons (1915), p. 117.

2. There is presented a series of data, assembled from various sources, which proves that in the visual discrimination of intensity the threshold difference ΔI bears no constant relation to the intensity I. The evidence shows unequivocally that as the intensity rises, the ratio $\frac{\Delta I}{I}$ first decreases and then increases.

3. The data are then subjected to analysis in terms of a photochemical system already proposed for the visual activity of the rods and cones. It is found that for the retinal elements to discriminate between one intensity and the next perceptible one, the transition from one to the other must involve the decomposition of a constant amount of photosensitive material.

4. The magnitude of this unitary increment in the quantity of photochemical action is greater for the rods than for the cones. Therefore, below a certain critical illumination—the cone threshold—intensity discrimination is controlled by the rods alone, but above this point it is determined by the cones alone.

5. The unitary increments in retinal photochemical action may be interpreted as being recorded by each rod and cone; or as conditioning the variability of the retinal cells so that each increment involves a constant increase in the number of active elements; or as a combination of the two interpretations.

6. Comparison with critical data of such diverse nature as dark adaptation, absolute thresholds, and visual acuity shows that the analysis is consistent with well established facts of vision.

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THE KINETICS OF STARVATION.

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(Accepted for publication, September 30, 1924.)

During the last few years an increasing number of data bearing on certain gross biological processes have been interpreted in terms of definite physicochemical laws. Robertson,[1] for instance, has shown that the growth of human infants may be represented by the curve of an autocatalytic monomolecular reaction. The growth of animals as well as the growth of plants has also been shown to conform to this law.[1] Osterhout,[2] furthermore, has illustrated the phenomena of injury, recovery, and death of tissues by means of equations which are characteristic of consecutive chemical reactions. More recently, Brody and his coworkers,[3,4] have made use of similar equations in studying the course of lactation in the dairy cow and the rate of ovulation in the fowl. To date, such analyses have not been applied to the phenomena of starvation.

In the course of experiments conducted in this laboratory to determine the value of certain vitamin-bearing substances the animals that were used were found to have undergone progressive inanition. When the relative weight loss of the series was plotted against time it was noted that the curve was similar to the growth curve of an infant. Accordingly, it was thought profitable to calculate the neces-

[1] Robertson, T. B., The chemical basis of growth and senescence, Monographs on experimental biology, Philadelphia and London, 1923.

[2] Osterhout, W. J. V., Injury, recovery, and death, in relation to conductivity and permeability, Monographs on experimental biology, Philadelphia and London, 1922.

[3] Brody, S., Ragsdale, A. C., and Turner, C. W., *J. Gen. Physiol.*, 1923–24, vi, 31.

[4] Brody, S., Henderson, E. W., and Kempster, H. L., *J. Gen. Physiol.*, 1923–24, vi, 41.

sary constants for the growth equation from the experimental data and then to compare the theoretical with the observed results in order to determine, if possible, the kinetic relationships in the process of starvation.

Method.

The data were obtained from a series of fifty adult pigeons of both sexes which were kept under approximately the same laboratory conditions over a period of 53 days. Rations consisted of a high grade of polished rice with sufficient water. A portion of the animals also received small doses of tomato juice. About one-third of the animals developed beriberi so that the juice was considered practically impotent in the doses administered. The birds were kept in newly built cages, at ordinary room temperature, with plenty of fresh air and daylight entering the quarters. They were weighed twice weekly, and the weights were recorded to the nearest 5 gm.

In the original experiment the weights of the animals were used as one of the measures of the potency of the vitamin substances given the "treated" animals. For this investigation the weights were taken as an index of the progress of starvation, just as they are used as an index of growth. The difference in the weights of the treated animals and the controls who received only rice and water was entirely insignificant. It was not only obvious that the entire group was undergoing starvation, but also that this common process was progressing at identically the same rate. It seemed quite proper, therefore, to consider all the animals as a single group, and to include the weights in a single series.

Although no exact measurement was made of the amount of rice consumed, it was evident that the quantities ingested could have had only a minor effect on the entire course of starvation. Pilcher,[5] working with pigeons under identical conditions in this laboratory, has weighed the amount of food eaten, and has found that it was dependent to a considerable extent on the amount of vitamin administered. From other experiments now being carried on it seems that the small amount of rice and vegetable juice served only to prolong

[5] Pilcher, J. D. (unpublished report).

the starvation period and not to alter the nature of the process. In the strictest sense, starvation was not complete. Yet, on the other hand, it was plain that the animals were receiving far less food than would maintain their weight.

Results.

1. Application of the Simple Autocatalytic Monomolecular Law.— In the third column of Table I is given the observed mean weight loss, expressed in per cent, of all the pigeons in the series over the 53 day period of the experiment. This set of data is graphically illustrated in Fig. 1 by the large dots. The broken line which meets and which is continuous with the smooth curve at the 21st day is the curve plotted from values of the weight loss calculated by means of the simple autocatalytic monomolecular law. The smooth curve is plotted from the values of an equation which has been derived as a modification of the autocatalytic law and which will be considered in detail later.

It will be seen on referring to Fig. 1, that the broken curve, which is understood to be coincident with the smooth curve after the 21st day, meets the observed values very closely from this day to the 53rd day, but that previous to the 21st day there is more and more divergence until the discrepancy becomes maximum at 0 days. The broken line is represented by the equation of an autocatalytic monomolecular chemical reaction[1,6]

$$\log_e \frac{x}{a-x} = K(t - t_1) \tag{1}$$

the differential form of which is given by

$$\frac{dx}{dt} = kx\,(a - x), \tag{2}$$

in which x is the amount of change at time t, a is the total amount of change accomplished, t_1 is the time when the reaction is one-half complete, or when $x = \frac{1}{2}a$, and, in which k is the velocity constant of the reaction.

[6] Mellor, J. W., Chemical statics and dynamics, London, 1909.

Three characteristics of the curve of this equation are evident, and it may be seen that the plotted values representing the observed weight loss also tend to lie along the same curve. They are (1) an initial period during which the slope of the curve gradually increases; (2) a point of inflexion situated approximately at the 21st day; and (3) a gradual diminution in the slope while the curve is approaching asymptotically to an upper limit of 50 per cent.

TABLE I.

Time.		Observed weight loss. x per cent.	K
days	*mos.*		
0	0.00	0.00	
5	0.16	3.70	1.96
10	0.33	10.60	1.46
15	0.50	16.90	1.33
18	0.60	20.20	1.40
21	0.70	24.40	1.01
24	0.80	27.60	1.14
26	0.87	29.80	1.12
29	0.96	31.60	0.97
32	1.06	34.60	1.03
35	1.17	37.00	1.01
38	1.26	39.20	1.04
40	1.33	40.50	1.03
44	1.46	42.50	1.02
47	1.57	44.00	1.02
50	1.66	46.30	1.16
53	1.76	47.50	1.20
			μ=1.06

a, taken approximately as 50 per cent.
T_1 = 0.72 month (when $x = \frac{1}{2}a$ = 25 per cent).
μ, mean value of K in last twelve observations.
P. E. of the series = ± 0.0469.

In order that the theoretical values of the curve could be determined it was first necessary to calculate K, the velocity constant. This could have been done by simply introducing the observed values of a, x, t, t_1, and solving for K directly, but by making use of tables prepared by Robertson[1] which gave the values of $K(t_1 - t_1)$ for any value $\frac{x}{a}$, the

work was considerably simplified. Table I shows the different values of K calculated by this method. The factor t_1 was found to be 0.72 month, since exactly one-half of the total 50 per cent loss had been transformed at this time. Simple division of $K(t - t_1)$ by the quantity $(t - t_1)$ estimated from the data gave a value for K at each recorded observation.

The results of Table I and Fig. 1 may be considered in detail. K as determined by the above method is seen to be relatively high

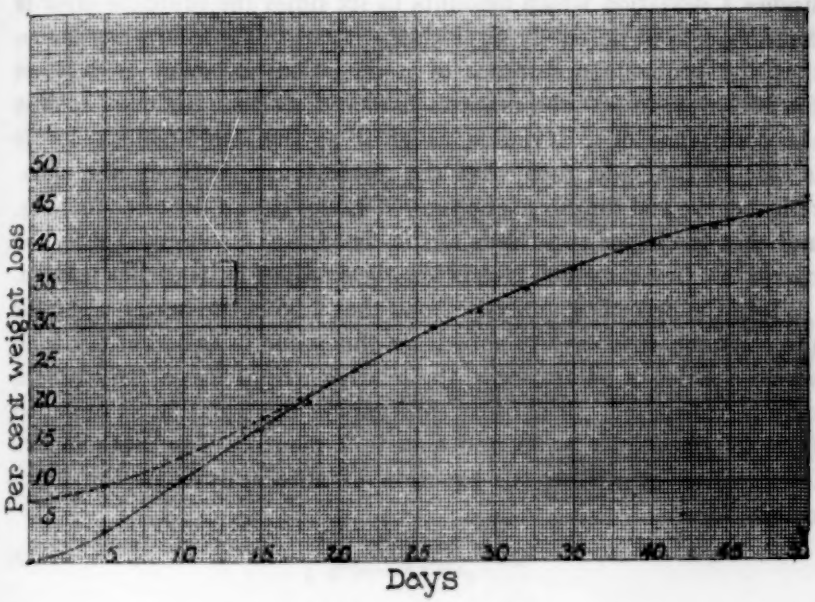

FIG. 1.

during the first 18 days of the experiment, but following this date the values of the constant are lower and show greater agreement. The mean of the whole series is 1.18; when, however, only those values after the 18th day are taken into consideration the mean is 1.06. The probable error, ϵ, of the series was obtained from the usual formula [7,8]

[7] Kelley, T. L., Statistical method, New York, 1923.

[8] Pearl, R., Introduction to medical biometry and statistics, Philadelphia and London, 1923.

$$P.E. = 0.6744\sqrt{\frac{\Sigma d^2}{n}} = 0.6744\sqrt{\frac{0.0581}{12}} = \pm 0.0469.$$

It is evident that all the deviations from the mean after the 18th day, with the exception of the last two deviations, are less than twice the probable error of the series. The last deviation is only 2.98 as large as the probable error. On the other hand, all of the values for K calculated from the date preceding the 18th day give deviations from 5.75 to nineteen times larger than the probable error. In biometric studies a deviation which amounts to six times the probable error is considered to be definitely significant. Pearl,[8] (Table 40), has shown that the probable occurrence of a deviation as small as 0.27 on the 15th day, in 100 observations is 0.0052, or that the odds against the occurrence of a deviation as great as this are 19,300 to 1. It is safe to conclude, then, that the discrepancies between the theoretical curve and the curve of observed values have a definite meaning, and that the latter two-thirds of the theoretical curve represents the course of a process which is significantly modified in its earlier portion.

Since the preceding analysis shows that 1.06 is the better value for the constant, it is easy to substitute this value in equation (1) from which a series of values for x, the relative weight loss at any time t, may be calculated. The other constants of the equation as obtained from the data are $a = 50$, and $t_1 = 0.72$ month. The mathematics is simplified by writing the equation of autocatalysis in the exponential form

$$x = \frac{ae^{2.3026\,K(t-t_1)}}{e^{2.3026\,K(t-t_1)} + 1} \tag{3}$$

in which e is the base of natural logarithms. Values for x so calculated are set down in Column 3 of Table III, and when plotted gave the broken curve in Fig. 1 already described.

In this instance again, it is seen that the values for the per cent weight loss are high during the early days of the experiment, but that after the 18th day there is substantial agreement between the observed and theoretical figures. Indeed, if the values at the beginning were to represent the true course of events, the birds weighed 7.72 per cent less than they actually did weigh at this time! Obviously, this is impossible; on the other hand, the agreement after the 21st day is suggestive.

From the above results alone it would be going too far to say, because of the agreement in the latter two-thirds of the starvation period, that the loss of weight followed the autocatalytic monomolecular law of chemical change. So far, only the relative weight loss has been considered because it was this curve that first attracted attention by its similarity to the growth curve. Instead of speaking in terms of relative weight loss it would probably simplify matters if a relation in terms of actual weights of the animals during the experiment could be derived.

2. *Derivation of the Weight Equation.*—It has been shown that the pigeons were losing weight in such a way that at the end of the experimental period the curve of relative weight loss was approaching an upper limit of 50 per cent. If then,

B_T denotes the weight at any time T,
B_0 " " initial weight which must be maximum,
T_1 " " time when the reaction is one-half complete, *i.e.* $B_T = \frac{1}{2} B_0$, or $x = \frac{1}{2}a$, according to equation (3),

the differential equation expressing the rate of loss may be immediately written if it is remembered that the lower asymptote of this curve is reached when $B_T = \frac{1}{2} B_0$ (50 per cent weight loss), and that the upper asymptote, of course, is limited by the value B_0. Furthermore, if this reaction is to be of the autocatalytic type, it must conform to the general equation

$$\frac{dx}{dt} = kx(a - x).$$

The derived equation would then be given by

$$-\frac{dB_T}{dT} = k(2B_T - B_0)(B_0 - B_T). \qquad (4)$$

When B_T is equal to B_0, the right-hand side of this equation passes to 0; it also passes to 0 when $2B_T$ is equal to B_0, so that at these points the slope of the curve $\left(\frac{dB_T}{dT}\right)$ is 0, and the conditions of the experimental curve are satisfied. The negative sign before the left-hand member shows that the value of B_T is diminishing as the value of T increases. The quantity $(2B_T - B_0)$ in equation (4) takes the place

of the quantity x in the general equation; equation (4), therefore, is of the type of equations expressing the rate of change of autocatalytic monomolecular reactions.

The above differential equation may be integrated most easily by the method of substitution:

$$-\frac{dB_T}{dT} = k(2B_T - B_0)(B_0 - B_T),$$

$$\int \frac{dB_T}{(2B_T - B_0)(B_0 - B_T)} = -k\int dT;$$

letting

$$2B_T - B_0 = u,\ B_T = \frac{u + B}{2};\ dB_T = \tfrac{1}{2}\,du;$$

substituting,

$$\int \frac{\frac{du}{2}}{\left(u + B_0 - B_0\right)\left(B_0 - \frac{u + B_0}{2}\right)} = -k\int dT.$$

Performing the indicated operations the equation becomes

$$\int \frac{du}{u(B_0 - u)} = -k\int dT.$$

This integral has been evaluated[9] and is

$$-\frac{1}{B_0}\cdot \log_e \frac{B_0 - u}{u};$$

resubstituting,

$$\log_e \frac{2B_0 - 2B_T}{2B_T - B_0} = k\,B_0 T,$$

which, on passing to common logarithms, becomes

$$\log_{10} \frac{2B_0 - 2B_T}{2B_T - B_0} = 0.4343 k B_0 T,$$

whence

$$\log_{10} \frac{2B_0 - 2B_T}{2B_T - 2B_0^{\frac{1}{2}}} = K(T - T_1) \qquad (5)$$

in which K is equal to $0.4343\ kB_0$.

[9] Peirce, B. O., Short table of integrals, Boston, 1889.

The exponential form of equation (5) is easily derived and is given by

$$B_T = \frac{B_0(e^{2.3026\,K(T-T_1)} + 2)}{2\,(e^{2.3026\,K(T-T_1)} + 1)}. \qquad (6)$$

By means of equation (6) the weights at different times were readily calculated with two settings on the slide-rule after the values of the exponential factor had been obtained from an appropriate table.[10]

TABLE II.

Time.	Observed weight.	Calculated weights.		
	M	B_T	A_t	M_T
mos.				
0.00	400	369	30.0	399
0.16	384	361	23.6	384
0.33	358	345	13.0	358
0.50	332	326	4.2	330
0.60	319	315	1.9	317
0.70	302	302	0.8	303
0.80	289	290	0.2	290
0.87	280	282	0.1	282
0.96	274	272	0.07	272
1.06	262	262	0.03	262
1.17	252	254		254
1.26	243	243		243
1.33	238	237		237
1.46	230	229		229
1.57	224	224		224
1.66	215	218		218
1.76	210	215		215

$A_0 = 30$ $\qquad T_1 = 0.72$ month
$B_0 = 400$ $\qquad k_1 = 3.94$
$t_1 = 0.30$ month $\qquad k_2 = 1.06$
P. E. of $M_T = \pm 1.26$

These results are found in Column 3 of Table II. They were based on an initial weight of 400 gm. for an adult pigeon, *i.e.* $B_0 = 400$. The plotted values of the weight curve represented by equation (6) lie along the broken curve in Fig. 2. Just as the broken curve of Fig. 1

[10] Hodgman, C. D., and Lange, N. A., Handbook of chemistry and physics, Cleveland, 1922.

was extended into the smooth curve at the 21st day, the broken curve of Fig. 2 also becomes continuous with its smooth curve at this time. The large dots again represent the observed values; the agreement between observed and calculated results after the 18th day is likewise apparent in this graph. In this instance, however, the theoretical curve falls definitely below the curve of observed weights, and by such a margin that a statistical analysis, similar to that employed in estimating the velocity constant, shows that the differences must certainly be significant.

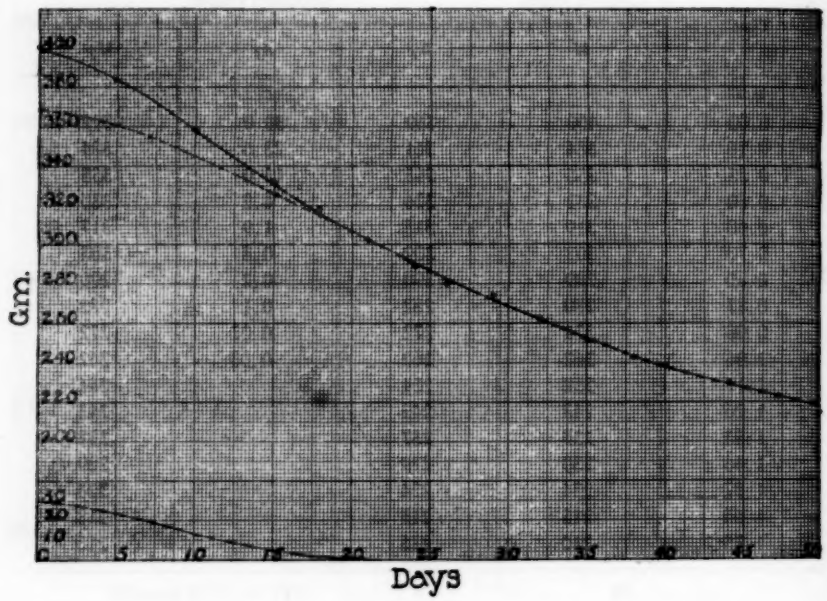

FIG. 2.

3. Corrections for Equations (3) and (6) with Final Results.—The discrepancies between theoretical and observed weights obtained from the preceding equations led to an hypothesis which will be discussed in the next section. Suffice it to say that a correction has been derived and applied to equations (3) and (6) which accounts for the differences during the early stages.

The corrected weight equation is equation (9); that for the relative weight loss is equation (10). Final theoretical values for the weight

M_T, were calculated by means of equation (9) and the results set up in the fifth column of Table II. These results are merely a summation of the values in Columns 3 and 4 of the same table. When the values M_T of the table were plotted they gave the smooth curve of Fig. 2. Table III shows the final results and corrected values of the relative weight loss m_T, which gave the smooth curve of Fig. 1. The agree-

TABLE III.

Time.	Observed weight loss.	Calculated weight loss.		
	m	b_T	a_t	m_T
mos.	*per cent*	*per cent*	*per cent*	*per cent*
0.00	0.00	7.72	7.25	0.47
0.16	3.70	9.92	6.05	3.87
0.33	10.60	14.00	3.35	10.65
0.50	16.90	18.50	1.10	17.40
0.60	20.20	21.40	0.48	20.92
0.70	24.40	24.50	0.20	24.30
0.80	27.60	27.50		27.50
0.87	29.80	29.50		29.50
0.96	31.60	32.00		32.00
1.06	34.60	34.70		34.70
1.17	37.00	37.50		37.50
1.26	39.20	39.20		39.20
1.33	40.50	40.80		40.80
1.46	42.50	42.90		42.90
1.57	44.00	44.00		44.00
1.66	46.30	45.50		45.50
1.76	47.50	46.20		46.20

a_o = 7.72 per cent T_1 = 0.72 month
b = 50.00 per cent k_1 = 3.94
t_1 = 0.30 month k_2 = 1.06
P. E. of m_T = $\pm$ 0.331

ment between the observed and calculated weights is now very close throughout the whole period of starvation.

DISCUSSION.

In the foregoing detailed analysis attention was called to certain discrepancies between observed and calculated results that prevailed

in the early part of the starvation period. Briefly, these were high values for the velocity constant of the reaction, high values for the relative weight loss, and low values for the actual weights. It was statistically shown that these discrepancies were more than a matter of chance, the significance of which was further augmented by the close agreement between theoretical and actual weights during the the remainder of the experiment. These facts, accordingly, led to the following interpretation of the results.

After the 18th day of starvation the pigeons were losing weight in accordance with the law of autocatalytic chemical change. Previous to this time, however, certain factors must have been influencing the rate of loss in such a manner that they prevented the reaction from proceeding as rapidly as it did later. Evidently, then, these inhibiting factors had been sufficiently diminished by the 18th day that their influence for practical purposes was negligible. It was plain that the birds had been undergoing a process of inanition relatively complete in the sense that they were receiving insufficient material of various kinds to enable them to maintain their adult weights. Immediate physiological demands as well as the preservation of life necessitated the utilization of all available food supplies exclusive of the small amounts of rice and vitamin that they were ingesting. These supplies could only have been obtained from their own storage depots and from their own body tissues. When the reserve stores had been exhausted their only remaining source of energy consisted of body protoplasm. Accordingly, the rate of loss of body weight after the inhibiting substances had been removed must have been proportional to the rate of destruction of body protein. But, it has been shown that after 18 days of starvation the rate of weight loss could be very closely represented by the equation of an autocatalytic monomolecular reaction. It seemed proper, therefore, to conclude that the destruction of body protein followed the law of autocatalysis.

Such a conclusion appeared justified in the light of Robertson's studies on the kinetics of growth,[1] in consequence of which he has interpreted growth as the final result of many processes primarily governed by the velocity of protein synthesis. Analogously, in the present instance, starvation was construed to be governed by the rate

of protein destruction, a process which was definitely modified in its earlier stages by the presence and by the destruction of storage substances—carbohydrate and fat, which, in turn, are known to prevent excessive protein loss. Many investigations on the metabolism of starvation apparently support this view. The protein-sparing action of carbohydrates and of fat has been substantiated by competent proof;[11] analyses of normal organs and of organs following starvation have shown that carbohydrate and fat are consistently destroyed in favor of protein;[12] the diminution in size of the body cells, the greater part of which is in the cytoplasm, has been frequently described in connection with the pathology of starvation.[13]

At first, it was thought that the preceding conditions might be expressed in terms of a consecutive chemical reaction.[2,6] Such a reaction is set forth symbolically by

$$X \rightarrow Y \rightarrow Z \rightarrow O$$

in which the initial substance X is undergoing change to an intermediate substance Y, which, in turn, is being transformed into a substance Z and so on. This equation, however, represents a reaction where some measure of the course of the reaction is increasing during the early stages, but where it is decreasing during the later stages. In the general equation above, a measure of this type of reaction would be contained in the factor Y or Z. The conductivity of tissues,[2] the rate of lactation of the dairy cow,[3] and the rate of ovulation of the fowl,[4] are examples of the application of the laws of consecutive chemical reactions to physiological problems. In each of these the index of the process at first increases with time to a maximum value after which it gradually diminishes.

From the above considerations it was at once evident that the phenomena of starvation under the conditions of the present experiment must be represented by a relation that is continually decreasing,

[11] MacLeod, J. J. R., Physiology and biochemistry in modern medicine, St. Louis, 3rd edition, 1920.

[12] Lusk, G., The elements of the science of nutrition, Philadelphia and London, 3rd edition, 1917.

[13] Adami, J. G., The principles of pathology, Philadelphia and New York, 2nd edition, 1910, i.

because in every case the initial weights were maximum. It seems quite possible that, at the very beginning, the process of growth and the process of starvation, measured by increase and by decrease of weight respectively, may have been in equilibrium, and, therefore, that any measure of the starvation process could be evaluated in terms of a consecutive reaction. Nevertheless, this period of equilibrium must have been decidedly short, and, accordingly, not subject to accurate verification. As the simplest explanation, then, these animals were considered to have been in equilibrium at the beginning of the experiment, from which the process of starvation took its origin.

TABLE IV.

Time.	y	k_1
mo.		
0.00	0.00	
0.03	1.30	4.9
0.16	7.00	3.7
0.33	17.00	3.8
0.50	25.00	3.5
0.60	28.00	4.1
0.70	29.00	3.6

A = 30.
t_1 = 0.30 month
Mean of k_1 = 3.94
Substance A assumed to be decomposing autocatalytically.
Note fairly constant value of k_1.

Consequently, it appeared highly probable that the resultant course of starvation, defined by weight of reacting tissue, could be much better expressed by the laws of simultaneous reactions than by those of consecutive reactions. Symbolically, again,

$$M = A + B, \tag{7}$$

in which M is a heterogeneous system composed of two independent homogeneous systems, A and B. Both A and B were assumed to be decomposing autocatalytically; A is taken as the factor of safety with respect to B, or, in the present case, the factor proportional to the amount of storage substances, and B is taken proportional to the

amount of primary reacting tissue. As a matter of fact, A was also assumed to be decomposing according to the simple monomolecular law, but when the constants were determined by the same methods as in the previous section, the agreement between the observed and calculated results was much closer in the former case. The monomolecular decomposition of A as calculated from the data is given in Table V and is shown by the broken curve in Fig. 3; the results of Table IV and the smooth curve of Fig. 3 illustrate the autocatalytic decomposition of A; the closer agreement of the latter results to the observed values is apparent.

TABLE V.

Time.	$A - y$	k
mo.		
0.00	30.0	
0.16	23.0	1.83
0.33	13.0	2.56
0.50	5.0	3.51
0.60	2.0	4.42

$A = 30.$

Mean of $k = 3.08$

Note increase in the value of k as reaction proceeds when the monomolecular equation $\frac{2.3026}{T} \log_{10} \frac{A}{A - y} = k$, is used.

On the basis of the above assumptions, which appear reasonably justified, it would follow that

$$\frac{dA_t}{dt} = ky(A_0 - y);$$

$$\int \frac{dA}{y(A_0 - y)} = -k \int dt;$$

integrating,

$$\log_e \frac{A_0 - A_t}{A_t} = k_1 t$$

or,

$$A_t = \frac{A_0}{e^{2.3026\, k_1(t - t_1)} + 1} \tag{8}$$

The equation representing the autocatalytic decomposition of B has been derived, and its value is given by equation (6) above. The general symbolical relation, (7), then becomes

$$M_T = \frac{A_0}{e^{2.3026\, k_1(t - t_1)} + 1} + \frac{B_0(e^{2.3026\, k_2(T - T_1)} + 2)}{2(e^{2.3026\, k_2(T - T_1)} + 1)}, \qquad (9)$$

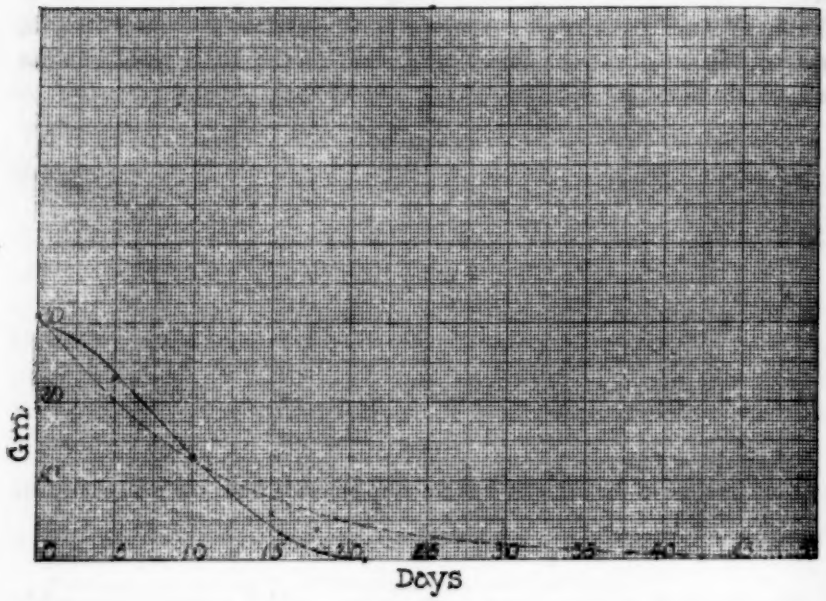

FIG. 3.

in which

M_T is proportional to weight of reacting tissue at time T,
A_0 " " " the initial amount of storage substances,
B_0 " " " " " " " primary body tissue,
T_1 " the time at which one-half of the reaction is complete,
t_1 " " " " " " " " storage substances are reduced,
e " " base of natural logarithms,
2.3026 " " reciprocal of the modulus of common logarithms,
k_1 " " velocity constant of the decomposition of A,
k_2 " " " " " " " " B.

The modified equation for the relative weight loss has been similarly derived. It is given by

$$m_T = \frac{b_0 e^{2.3026\, k_2 (T - T_1)}}{e^{2.3026\, k_2 (T - T_1)} + 1} - \frac{a_0}{e^{2.3026\, k_1 (t - t_1)} + 1}, \tag{10}$$

in which the units chosen represent relative weight loss and in which the constants have the same significance as above.

When the modified equations (9) and (10) were used to calculate the theoretical values for the weight and for the relative weight loss respectively during the entire course of the reaction, the results set up in Tables II and III were obtained; in Figs. 1 and 2 these values are represented by the smooth curves, which in both cases meet the observed values throughout the course of starvation with satisfactory precision.

In considering the relative rates of decomposition of substances A and B, it is important to note that while substance A is diminishing from a finite value of A_0 to a value that approaches 0 during its own time interval t, the substance B, on the other hand, is being transformed from a maximum value of B_0 to a value of B_C during the whole interval T. B_C is taken proportional to the value of the reacting mass of tissue at death. The ratio, $\frac{B_C}{B_0}$, therefore, is proportional to the remaining relative amount of tissue when the reaction is complete, and the ratio, $\frac{B_0 - B_C}{B_0}$, proportional to the maximum relative change that occurs, which, in the present experiment, as shown, is equal to 50 per cent.

From the experimental point of view it is of interest to note that, in those pigeons which develop beriberi on a rice diet, the onset of the disease usually falls within the 18 to 21 day period of starvation[14] at precisely the time when, as shown above, one-half of the process of starvation has been completed.

[14] Stepp, W., *Ergebn. Inn. Med. u. Kinderheilk.*, 1923, xxiii, 66.

SUMMARY AND CONCLUSIONS.

1. The relative weight loss of pigeons on starving rations may be represented by a simple modification of the law of autocatalysis.

2. An equation giving the actual weight of the animals during the 53 day period of the experiment is derived by means of the hypothesis that the process of inanition is governed by the simultaneous velocity relations between two independent, homogeneous systems, such that

$$M_T = (A_0 \rightarrow 0) + (B_0 \rightarrow B_C)$$

in which A is taken proportional to the amount of carbohydrate-fat reserves, and B proportional to the amount of reacting body protoplasm.

3. The course of starvation is governed by the rate of destruction of body protein, but it is modified by the amount and by the rate of destruction of reserve materials.

4. The processes of breaking down tissues are analogous to those by which tissues are synthesized.

5. The close agreement between observed and calculated values suggests that the original assumptions set forth in deriving the foregoing equations were valid.

6. Attention is called to the fact that the time of onset of beriberi in pigeons is coincident with the half period of starvation.

STUDIES ON THE FORMATION AND IONIZATION OF THE COMPOUNDS OF CASEIN WITH ALKALI.

I. The Transport Numbers of Alkali Caseinate Solutions.*

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(Accepted for publication, October 1, 1924.)

INTRODUCTION.

The ability of proteins to take up hydrogen ions from acid solutions and hydroxyl ions from alkaline solutions and the phenomena accompanying this, such as imbibition of water, have been explained from two very different view-points. Pure colloidal or adsorption theories (1, 2) have on the one hand received consideration and on the other hand what may be called chemical theories have been advanced to explain these phenomena. Although a great deal of experimental work which is favorable to the latter view has been brought forth, there is however no unanimity of opinion with respect to the groups within the protein molecule which enables it to combine with acids and with bases. It has been suggested by Loeb (3) and others (4–6) that this combination takes place through the agency of the free amino and the free carboxyl groups and the resulting compounds will therefore ionize to yield protein ions which will be positively or negatively charged depending on whether the protein is combined with an anion or a cation. A criticism of this theory lies in the fact that the proponents of this view have failed to show the presence within the protein molecule of a sufficient number of free amino or carboxyl groups in order to establish quantitative relationships.

* Aided by a grant from the Research Board of the University of California.

The theory proposed by Robertson disagrees with the above propositions and instead postulates that the $-COHN-$ groups of the protein molecule are the chief agencies which enable it to combine with both acids and bases and that complex protein ions are formed as shown by the following equations.

With acids,

$$-COH=N- + \overset{+}{H}\overset{-}{A} \rightarrow -\overset{++}{C}OH + \overset{\bar{}}{\underset{|}{\overset{H}{\underset{|}{N}}}}- \qquad (1)$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad A$$

With bases,

$$-COH=N- + B\,OH \rightarrow -\overset{++}{C}OB + \overset{\bar{}}{\underset{|}{\overset{H}{\underset{|}{N}}}} \qquad (2)$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad OH$$

EXPERIMENTAL.

From considerations of his theory Robertson concluded that the migration velocity of both the cation and anion of a protein salt should be equal; or in other words, the fraction of the current carried by each ion is one-half. Robertson (8) and later Haas (9) carried out experiments to test this hypothesis and concluded that their data confirmed the above hypothesis. The experimental method employed by Robertson and Haas consisted in electrolyzing casein solutions which had been placed in a two-compartment cell and then determining the amount of casein which was lost from each compartment. From theoretical considerations Robertson decided that if each ion carries one-half of the current the ratio of loss of casein from the anode compartment to that in the cathode must be as 2:1. Using solutions of potassium caseinate he, and Haas, did obtain this ratio. Robertson considered this as strong evidence in favor of this theory.

We decided that a direct determination of the transference numbers of protein solutions would be far preferable, and would give much stronger evidence for or against Robertson's theory than the experi-

ments described by him. Robertson's method of experimentation is not a standard method such as the well known methods for direct transference determination; and moreover, since no attempt was made to maintain a constant pH in the cathode compartment, and since the ionic properties of protein solutions are a function of the pH, comparable experimental data cannot be obtained when the pH is allowed to vary.

Accordingly, an examination was made of the methods in use in search of one which would suit our purpose. First, the moving boundary method of Dennison and Steele (10) was tried, but being unable to get a sharp boundary with protein solutions it was abandoned. We then tried the Hittorf method of direct analysis, and were able to work out a procedure that gave satisfactory results.

Since the ionic properties of a protein are a function of the pH it was necessary to employ a method which would maintain a constant pH during the course of the experiment. This was accomplished by the use of non-polarizable electrodes. To be sure that what we were measuring were the transport numbers of the casein ions, it was very desirable to determine directly the transport numbers of the alkali metal in the solution. This was accomplished by working out a titration method of determining the amount of alkali metal.

The cell which was used in the experiments is illustrated in Fig. 1. It is a modified form of the type of cell used by Washburn (11) in his very exact measurements of transference numbers. Essentially it consists of three compartments of approximately 60 cc. each, with stop-cocks which can be closed in order to keep the three portions from mixing at the close of an experiment. This type of cell proved very satisfactory. It is compact, the stop-cocks make it easy to separate the three portions from each other, and the cross section is large, a consideration which is very important since the protein solutions have a rather high electrical resistance which otherwise makes it difficult to pass sufficiently large currents through the solution. For the cathode, an electrode of Pb covered with PbO_2 was used. This keeps the pH constant in the cathode portion and no evolution of hydrogen was observed. For the anode either a spiral of platinum wire or a piece of platinum gauze was used. With an alkali caseinate

solution, on the passage of a direct current, casein is deposited on this platinum electrode giving a non-polarizable electrode that maintains the pH practically unchanged in the anode portion. This observation was first made by Robertson. The method, however, seems to be only applicable to proteins which are insoluble at their isoelectric points. We tried the method on gelatin solutions, adding small quantities of alkali from time to time to maintain an approximately constant pH but the results obtained were never concordant.

The casein used was made according to the procedure of Van Slyke and Baker (12) with some slight modification. After the casein was precipitated from the skimmed milk, it was put into tall

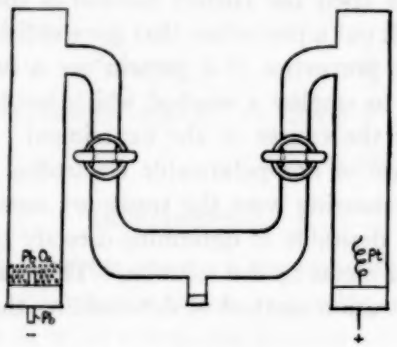

FIG. 1.

glass cylinders and washed with distilled water by decantation several times a day for a period of 2 weeks or more. As a test for completeness of washing the wash waters were tested for chloride ion. After this the material was dried by first washing two or three times with 95 per cent alcohol, several times with absolute alcohol, and finally a number of times with absolute ether. In this way all but a trace of fat was also removed. Finally the ether was drained off and the casein was dried in an oven over H_2SO_4 at 40°C. A pure, snow-white, finely divided material was obtained. One lot was made by drying with acetone instead of ether, but the casein obtained was colored somewhat yellow and gave dark solutions which were more viscous than those in which the casein was obtained by drying with ether.

The object of using racemic casein and the method used for its preparation will be discussed in the third paper of this series. In all the transference and conductivity determinations on racemic casein, the sample with 13.5 per cent N was used. During the course of the work casein was prepared a considerable number of times, and while different samples gave some differences in the values which were obtained, especially of the electrochemical equivalent, still the results are as a whole in strikingly good agreement. An analysis of the ash of the casein gave 0.4 and 0.3 per cent, respectively, for one sample of the Van Slyke and Baker casein, and 0.6 and 0.5 per cent for the racemic casein.

TABLE I.

Re-solution of Casein from the Electrode.

Casein.	Base.	Time.	Q for electrode left standing.	Q for other electrode.	Loss per hr.
per cent	*cc.*	*hrs.*			*per cent*
2.17	5.15 (CsOH)	1.5	1.74	1.72	0
1.93	5.00 (KOH)	2	2.09 2.05	2.06 2.04	0
1.96 (racemic casein).	7.47 (NaOH)	1	1.17	1.18	0.85
2.17 (racemic casein).	10.2 (KOH)	2	0.873 0.860	0.910 0.918	2.5

The analysis for casein in these experiments was made by evaporating 10 cc. portions of the solution, drying at 100°C. in an electric oven, and weighing the residue. From this weight, the weight of alkali metal in the solution was subtracted. As a check on this method, in one experiment the casein was analyzed by determining the nitrogen; both methods gave the same results within the limits of experimental error. The method of determining the amount of alkali metal in solution consists simply in titrating with a strong acid to the isoelectric point of casein. We used 0.05 N trichloroacetic acid, which gives very good results. The casein acts as its own indicator. As the isoelectric point is approached the solution becomes very opaque, but there is no settling out of protein particles, probably

due to their still being electrically charged. On the addition of a small additional amount of acid, the isoelectric point is reached. At this point, whirling or stirring the solution causes the casein to coalesce into large particles which rapidly sink to the bottom leaving the liquid above nearly transparent. The effect is very striking and is an indicator which makes it easy to determine when the end-point is reached. This method seems applicable to all proteins which are insoluble at their isoelectric point. Good results were obtained with gliadin solutions. The method can probably be used also for determining the amount of acid in an acid-casein solution by titrating with an alkali. It has already been stated that during the passage of the current casein is deposited on the anode. Previous to this investigation, Robertson was the only one who had quantitatively investigated the phenomenon. He interpreted it as follows:[1]

"We have seen that the casein anion, which is free from base, must migrate to the anode. There it may be presumed to react with water, liberating oxygen and free casein, which combines with the excess of base until the proportion of base to casein in the film in immediate contact with the anode falls to that which obtains at 'saturation' of the base with casein. Any additional casein thus migrating into the film in contact with the anode must be precipitated as uncombined casein. . . . . Hence the electrochemical equivalent which is actually measured in solutions of all reactions is that of casein at 'saturation' of the base with the protein."

We studied the electrode deposition of casein in connection with our transference determinations and have come to quite a different conclusion. Pauli (5) also criticized Robertson's conclusions and experimental methods, and from theoretical considerations arrived at a belief which is very much in accord with our own results. From our experimental work we concluded that the amount of casein deposited is strictly proportional to the amount of current which passed through the solution; *i.e.*, Faraday's law holds for these solutions.

An examination of Table II shows that Q, the electrochemical equivalent, is the same for solutions with equivalent amounts of alkali per gm. of casein, and is independent of the alkali used (Na, K, Rb, Cs), or the size and shape of the electrodes. However, instead of Q being constant and being the electrochemical equivalent at

saturation of base with casein, we found that Q does vary with the amount of alkali in solution. We found that Q obeys the relationship:

$$Q \times B = K$$

TABLE II.

*Transport Numbers (Average Values at 30°C.).**

Time.	Casein.	B approximately.	pH	Q	K	Casein transferred per millifaraday.	T_{casein}	T_{Na}
hrs.	*per cent*	*cc.*						
				(a) Sodium Caseinate Solutions.				
2.5–3	1.75–3.0	5.6	6.6	1.73	9.68	0.784	0.453	0.561
2.5	2.5	7.0	7.0	1.34	9.40	0.610	0.455	0.540
2.5	1.7	8.25	7.6	1.04	8.60	0.450	0.430	
				(b) Potassium Caseinate Solutions.				
3	2–3	5	6.5	1.82	9.09	0.650	0.359	0.660
2.25–4.0	2.5–3	6.25	6.9	1.47	9.17	0.534	0.363	0.636
2–2.5	2–2.5	8.0	7.6	1.12	9.00	0.390	0.349	0.657
2.0	2.0	10.0	9.4	0.92	9.20	0.350	0.382	0.654
				(c) Rubidium Caseinate Solutions.				
2.0	1.8	5.6	6.5	1.76	9.86	0.625	0.355	0.645
				(d) Cesium Caseinate Solutions.				
2–2.5	2.0	5.5	6.5	1.65	9.10	0.551	0.334	0.666
				(e) Sodium Racemic Caseinate Solutions.				
3–3.5	2.0	5.4	5.75	1.63	8.83	0.635	0.390	0.610
				(f) Potassium Racemic Caseinate Solutions.				
2.25–3.0	2–2.5	6.0	5.5	1.46	8.76	0.465	0.318	0.680
1.5 –2.5	1.6	8.65	6.4	0.985	8.50	0.355	0.360	0.610

B = cc. 0.1 N. alkali per gm. casein.

Q = electrochemical equivalent per millifaraday.

* Lack of space prevents publication of the data in detail.

in which Q is the electrochemical equivalent per millifaraday, B is the number of cc. of 0.1 N alkali per gm. of casein, and K is a constant. In other words, the electrochemical equivalent is inversely proportional to the amount of alkali which has combined with the casein. In the solutions with which we worked, the total amount of alkali can be considered as combined with the casein without making any appreciable error. An examination of Table II shows that while B has been varied between 4.8 cc. of 0.1 N alkali per gm., to over 10 cc. of 0.1 N alkali the value of K remains essentially the same.

It is true that the values of K which were obtained with solutions of higher alkali content are somewhat lower than the average values of K, obtained when B is between 5 and 6 cc. of 0.1 N alkali per gm. This is to be accounted for by a dissolving off of part of the casein from the electrode during the course of the experiments. The higher the alkalinity, the more rapid this re-solution. Robertson noticed this re-solution and attempted to determine the amount lost by re-solution from the electrode in the solutions with which he worked. We also made some attempts to determine the loss of casein from the electrode. To maintain the conditions as nearly as possible the same as during the passage of the current, we electrolyzed samples of the same solution in each of two cells, and at the end of the electrolysis we let the electrodes stand in one cell, just as it was, for a period of several hours; while in the other cell we removed the electrode, and washed and dried it at once. In this way, from the difference in Q we could tell how much casein was lost from the electrode. The results are given in Table I. It is to be seen that in low concentrations of alkali, between 5 and 6 cc. of 0.1 N alkali per gm., the loss is negligible. In higher concentrations of alkali the loss does become significant.

In Fig. 2 are given curves for K plotted against B (cc. of 0.1 N alkali per gm.), for casein and racemic casein. The values of K as plotted are averages, and are uncorrected with respect to re-solution of casein from the electrode. It is seen that the experimental points fall on both sides of the theoretical curve which appears to be a straight line parallel to the abscissa. It will be noticed that when the alkalinity rises above pH 7 the points fall below the theoretical curve. This probably indicates a small amount of re-solution of the

casein. The magnitude, however, is well within the maximum experimental error.

We can obtain a very interesting check on the value of K from an entirely different source. Cohn and Hendry (13) have published a very accurate study of the solubility relations of casein in NaOH. They found that at saturation with casein it takes 4.76 cc. of 0.1 N NaOH to dissolve 1 gm. of casein, which gives an equivalent weight of casein of 2,100. From the values of K which we have determined, the equivalent weight of casein can also be calculated at the value

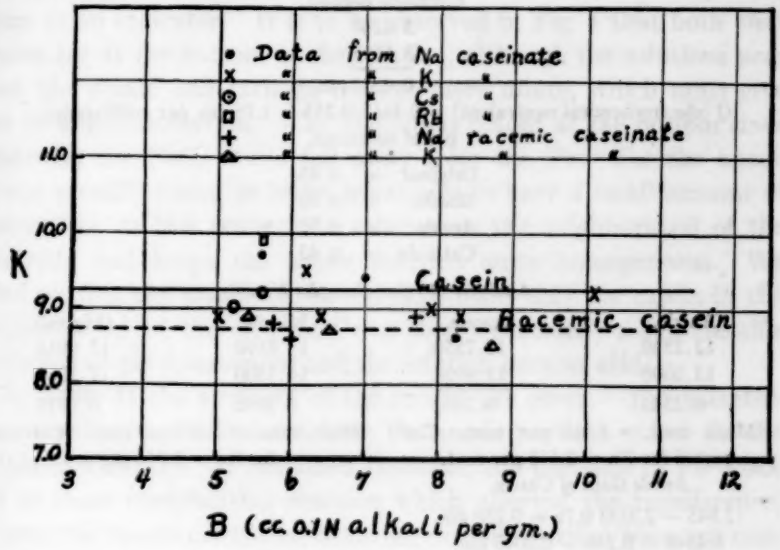

FIG. 2.

of 4.76 cc. of 0.1 N alkali per gm. of casein; and doing so we obtain $1{,}000 \times Q = \left(\frac{9.6}{4.76}\right) 1{,}000 = 2{,}015$ gm. This is a good agreement considering the great difference in the methods which have been employed.

Having now discussed the meaning of the electrode deposit of casein, we can again take up the determination of transference numbers. The electrode deposit is an important quantity in these determinations, as it is a measure of the current which has passed

through the solution. Since our knowledge of the equivalent weight of casein is otherwise very uncertain, calculation of the transference

TABLE III.

Calculation of Transference Experiments.

Sample A.

2.85 per cent casein + 5.6 cc. 0.1 N NaOH per gm. Temperature 30°C. Time 2.5 hrs. Current 2.55 cc. 0.1 N Ag.

Anode Portion = 76 Gm.

Electrode deposit.

3.6240
3.1695
0.4545 gm.

Q (electrochemical equivalent) = 0.4545/0.255 = 1.78 gm. per millifaraday.

pH of solutions.

Original = 6.45
Middle = 6.50
Anode = 6.50
Cathode = 6.45

Analysis (10 Gm. Samples Used).

Anode.	Anode.	Middle.	Original.
13.2540	12.7550	15.0190	17.9913
13.0000	12.4998	14.7300	17.7035
0.2540	0.2552	0.2890	0.2878

Mean conc. = 2.546 per cent. Corrected for Na = 2.515 per cent.

Mean conc. = 2.884 per cent. Corrected for Na = 2.845 per cent.

Anode Gain of Casein.

(2.845 − 2.515) 0.76 = 0.250 gm.
0.4545 − 0.250 = 0.2045 gm.

Transfer of casein per millifaraday.*

0.2045/0.255 = 0.802 gm.

Anode Loss of Na.

(15.9 − 13.9) 0.76 = 1.52 cc. 0.1 N Na

T_{casein}^{-} = 0.802/1.78 = 0.45

T_{Na}^{+} = 1.52/2.55 = 0.596

Total..... (0.45 + 0.596) = 1.046

Alkali Analysis (Titration with Trichloroacetic Acid).

Amount.	Sample.	0.05 N acid.	0.1 N per 100 gm. sample.
cc.		cc.	cc.
40	Middle.	12.8	15.9
40	Anode.	11.1	13.9

* Since each cc. of 0.1 N Ag liberated in the coulometer represents 0.1 millifaraday then 2.55 cc. is equivalent to 0.255 millifaraday.

numbers of the casein ion, assuming some value for the equivalent weight, would give doubtful results. But knowing the change in concentration of casein in the anode portion, and the weight of casein deposited on the anode, the transference numbers of the casein ion can easily be calculated.

The methods of analysis used have already been described. It is only necessary to add further that the amount of current was determined with the aid of a silver titration coulometer, the dissolved silver being titrated with 0.025 N thiocyanate solution using ferric alum as an indicator. It is to be observed in Fig. 1 that both electrodes are at the bottom of the solution, although the solutions near both the anode and cathode become more dilute, which must give rise to some convection. The cathode is placed at the bottom since otherwise the PbO_2 would fall away from the Pb. For the anode it was actually found to be an advantage to have a small amount of convection, as this renews the solution in the neighborhood of the electrode and keeps the anode solution quite homogeneous. We tried putting the anode at the top and found that the casein in the neighborhood of the electrode was soon exhausted; a considerable evolution of gas commenced and the solution became acid.

In Table II the averages of the results are given. The transference numbers are calculated from the anode portions only. In the cathode, while the pH remained constant, the presence of Pb–PbO_2 led to some complicating reaction which affected the transference, so that the values calculated from the cathode portion were not concordant. To illustrate how the calculations of the transference numbers were made, a sample note-book page is given in Table III and the calculations are shown.

The transport numbers for the casein are obtained from the electrode deposit and the total gain of casein in the anode portion. The transport number of the cation is calculated from the determination of the quantity of electricity and the loss of cation in the anode portion. It is to be seen from the tables that the sum of the two is always close to unity. This favors the view that we are really measuring the transport numbers. In these solutions the alkalinity was always negligibly small and no correction was needed.

According to Robertson's theory,[1] the transport number of both anion and cation, as we have stated before, should each be one-half. In the tabulated results it is seen that we did not obtain this figure. Instead, the values of the transport numbers vary according to the mobility of the cation which was combined with the casein. That this is so is evident from the following considerations. The transport number of an ion, is given by the equation

$$\text{anion} = \frac{\Lambda_{0\ \text{anion}}}{\Lambda_{0\ \text{anion}} + \Lambda_{0\ \text{cation}}},$$

TABLE IV.

Mobility of Casein Ion Calculated from Transference Numbers.

Cation used.	$T_{\bar{\text{casein}}}$	30°C. $\Lambda_{0\text{cation}}$	30°C. $\Lambda_{0\bar{\text{casein}}}$	30°C. $\Lambda_{0\text{compound}}$
Casein.				
Sodium	0.453	56.2	46.2	102.4
Potassium	0.363	81.4	46.5	127.9
Cesium	0.334	85.3	43.0	128.3
Rubidium	0.355	84.5	45.5	130.0
Racemic casein.				
Sodium	0.390	56.2	37.0	93.0
Potassium	0.318	81.4	37.7	119.1

in which Λ_0 is the mobility or equivalent conductivity at infinite dilution. We have the data for the transport numbers from our experiments, and data for the mobility of the cations used can be obtained from collected tables of physical constants such as the Landolt-Börnstein *Tabellen* (14). With the aid of these we can solve for the mobility of the anion, which in this case will be the casein ion. Now if the transport numbers vary according to the mobility of the cation used, the value calculated for the mobility of the casein ion should be the same in all cases. The results of these calculations are given in Table IV.

[1] Robertson (7), p. 180.

The transference numbers of the casein solutions containing between 5 and 6 cc. of 0.1 N alkali per gm. casein are used for the calculations, as more determinations were carried out with these. The values of the mobilities at 18°C. for Na^+, K^+, Cs^+, and Rb^+, and their temperature coefficients were taken from the Landolt-Börnstein *Tabellen*,[2] and from them the values of the mobilities for 30°C. were calculated. It is to be seen there is a surprisingly good correspondence in the values which we obtained for the mobility of the casein ion, especially since all the errors are thrown on the values for the mobility of the casein ion. The value obtained for the mobility of the racemic casein is about 20 per cent less than that of ordinary casein. Why this should be we have for the present no explanation.

Using these results we can now explain why Robertson (8) and Haas (9) obtained the values which they found in their migration experiments. It is to be remembered that, using two-compartment cells and electrolyzing casein solutions, they found the ratio of loss from the anode compartment to that of the cathode compartment to be in the ratio of 2:1. They used only potassium caseinate solutions. We have found that the transport number of the caseinate ion in potassium caseinate solution is 0.363. As a first approximation we will call it $\frac{1}{3}$. During the passage of the current a certain amount of casein will be deposited on the anode electrode, which will be a measure of the current. At the same time there will be a migration of casein from the cathode to the anode. Now if we call the casein deposited a, the amount moving from the cathode to the anode will be $\frac{1}{3}\ a$. The amount lost from the anode portion will be $(a - \frac{1}{3}\ a = \frac{2}{3}\ a$. The amount lost from the cathode portion will be $\frac{1}{3}\ a$ and the ratio of loss will be as 2:1. It is only because potassium caseinate was used that Robertson, and Haas, obtained these results. Had they used sodium caseinate, it is safe to say that they would have found an entirely different ratio of loss. It might be asked why the 2:1 ratio was obtained, since the transference number of the caseinate ion is 0.363 and not $\frac{1}{3}$. The answer probably is that since no attempts were made to maintain a constant pH in the cathode compart-

[2] Landolt-Börnstein (14), p. 1104.

ment, hydroxyl ions accumulated which took part in carrying the current and thus lowered the transference number of the caseinate ion.

Another very important point is the high mobility calculated for the casein ion. This will be discussed in a second paper, where it is considered in relation to conductivity determinations made by us and by others with alkali caseinate solutions.

SUMMARY.

1. The deposition of casein on a platinum anode which takes place on the passage of a direct current through solutions of alkali caseinates was quantitatively studied, and it was found that: (*a*) the amount of casein which is deposited is directly proportional to the current, *i.e.* it obeys Faraday's law; (*b*) the amount of casein deposited is inversely proportional (within the limits studied) to the amount of alkali which is combined with the casein.

2. A method of determining the transport numbers of proteins insoluble at their isoelectric point has been developed.

3. A titration method for determining the amount of alkali in a casein solution is given.

4. Data from the results of transference experiments on sodium caseinate, potassium caseinate, cesium caseinate, and rubidium caseinate solutions are given. It is shown that the data are best explained on the assumption that in these solutions the carriers of the current are alkali metal cations and casein anions.

5. On the basis of our transference results an explanation is given of the results which were obtained by Robertson and by Haas in their migration experiments.

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STUDIES ON THE FORMATION AND IONIZATION OF THE COMPOUNDS OF CASEIN WITH ALKALI.

II. The Conductivities of Alkali Caseinate Solutions.*

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(Accepted for publication, October 1, 1924.)

Conductivity Results.

In this paper we shall attempt to show that the results of conductivity experiments with alkali caseinate solutions support the conclusions at which we arrived from transference experiments described in the previous paper.

All the determinations which were made by us were carried out according to the well known Kohlrausch method. An oscillator with a tuning fork (one thousand vibrations per second) was used for the source of current, and the readings were made with the aid of a Leeds and Northrup slide-wire bridge and telephone. The platinum electrodes were coated with platinum black. The solutions which were used in these experiments were analyzed for casein and for alkali concentration as described in the previous paper. The water which was used had an average conductivity of 3×10^{-6} mhos.

A number of investigators have determined the conductivity of alkali caseinate solutions (Laqueur and Sackur (1), Robertson (2), and quite recently Pauli and Matula (3)). It is interesting to compare the results of the different investigators, including those obtained by us. In Table I there are collected certain of the conductivity data at comparable concentrations. Although the results are all of the same order of magnitude they vary considerably among themselves. It is also to be observed that the different series of our own

* Aided by a grant from the Research Board of the University of California.

determinations vary among themselves by as much as 10 per cent. However, by taking the averages and discarding the values which vary widely from the mean, values are obtained which are probably correct within a few per cent, plus or minus. This statement will be justified when the extrapolation of our data to infinite dilution is

TABLE I.

Comparison of Conductivity Data (25°C.).

Casein + approximately 5 cc. 0.1 N NaOH per gm.

Alkali concentration in equivalents.	Equivalent conductivity.				
	I	II	IV	IV	IV
	pH = 6.3		pH = 6.5		
0.02		37.5	51.7	46.8	47.0
0.01	50.6	41.7	56.9	51.5	53.5
0.005	55.4	46.5	62.9	57.0	57.6
0.0025	59.6	52.3	64.8	65.0	68.0
0.00125	67.2		74.0	76.5	69.2

Casein + approximately 8 cc. 0.1 N NaOH per gm.

Alkali concentration in equivalents.	Equivalent conductivity.		
	I	III	IV
			pH = 7.5
0.025		46.5	
0.010	52.7	52.3	54.0
0.005	57.4	57.2	59.0
0.0025	64.8	64.0	64.0
0.00125	72.0	71.0	

I = experimental data of Pauli and Matula (3).
II = " " " Robertson (2).
III = " " " Laqueur and Sackur (1).
IV = taken from our data.

considered. It is rather gratifying to find that our average results correspond rather well with those of Pauli, which are the most recent of those cited in the literature.

When one considers that the accuracy of the Kohlrausch method for the determination of the conductivity of electrolytes ordinarily lies within 1 per cent, it appears that some factor which at present

is not obvious enters into the method when applied to protein solutions. About 20 years ago Hardy (4) claimed that the use of platinum black electrodes led to inaccuracies in estimating the conductivity of protein solutions; but Robertson (2) considers that he has been able to overcome the objections raised by Hardy. However, our own work, as well as the work of others, points to the need of a reinvestigation of this subject.

The problem of extrapolating the conductivity data to infinite dilutions is a complex one. The degree of ionization is not nearly as large as in such salts as potassium or sodium chloride. To determine the conductivity in very dilute solutions offers many difficulties. The only other alternative lies in the possibility of discovering a dilution law which is obeyed by these solutions, and of solving either analytically or graphically for the value at infinite dilution. Many such empirical methods have been suggested for strong electrolytes. All of these are essentially modifications of Storch's (5) equation. For the purpose of plotting, this may be put in the form

$$(C\,\Lambda)^n = f\left(\frac{1}{\Lambda}\right)$$

By plotting some power of $C\Lambda$ against the reciprocal of the equivalent conductivity, the value of Λ_0 is obtained by prolonging the curve obtained to the point where $(C\,\Lambda)^n = 0$; at that point $\frac{1}{\frac{1}{\Lambda}} = \Lambda_0$. Of course this can be done with the greatest degree of certainty when the graph obtained is a straight line; so that generally it is sought to use such a value of n as will give a straight line.

Pauli and Matula (3) in their conductivity studies of alkali caseinates, attempt to extrapolate their data by plotting the concentration against the equivalent conductivity. This unfortunately does not give a straight line, which introduces a good deal of uncertainty in their extrapolation. Mindful of the fact that for many strong electrolytes plotting $(C\Lambda)^{\frac{1}{2}}$ against $\frac{1}{\Lambda}$ gives a straight line, we plotted our data in this way and found the resulting graph to be a straight line.

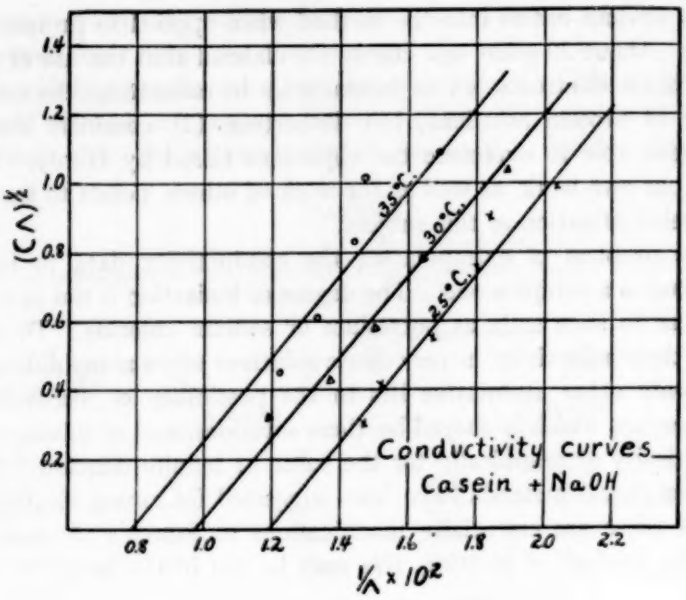
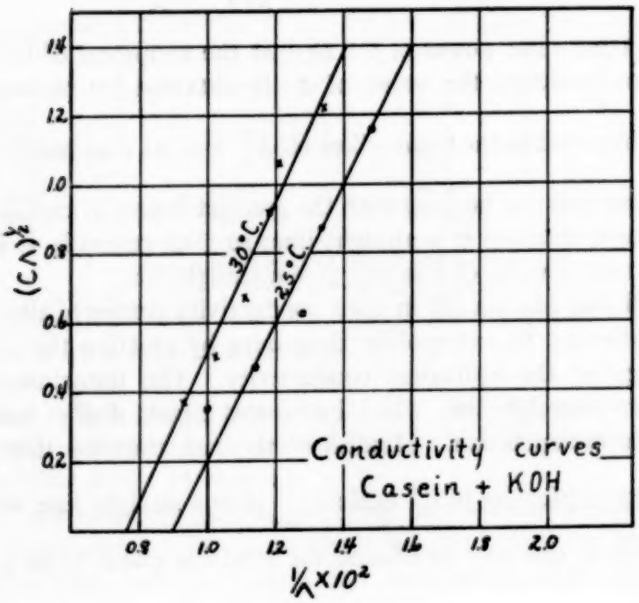

1.4
1.2
1.0
0.8
0.6
0.4
0.2
$(C\Lambda)^{\frac{1}{2}}$
35°C.
30°C.
25°C.
Conductivity curves
Casein + NaOH
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
$1/\Lambda \times 10^2$

FIG. 1.


1.4
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$(C\Lambda)^{\frac{1}{2}}$
30°C.
25°C.
Conductivity curves
Casein + KOH
0.8
1.0
1.2
1.4
1.6
1.8
2.0
$1/\Lambda \times 10^2$

FIG. 2.

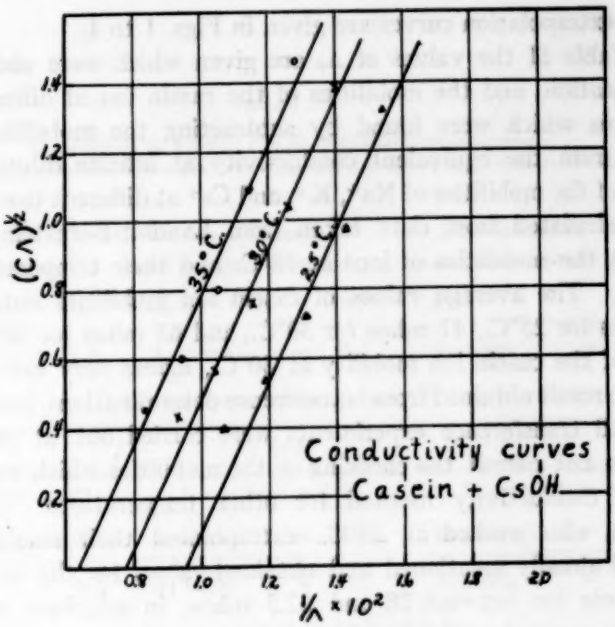
1.4
1.2
1.0
0.8
0.6
0.4
0.2
(CΛ)½
35°C.
30°C.
25°C.
Conductivity curves
Casein + CsOH
0.8
1.0
1.2
1.4
1.6
1.8
2.0
1/Λ × 10²

FIG. 3.

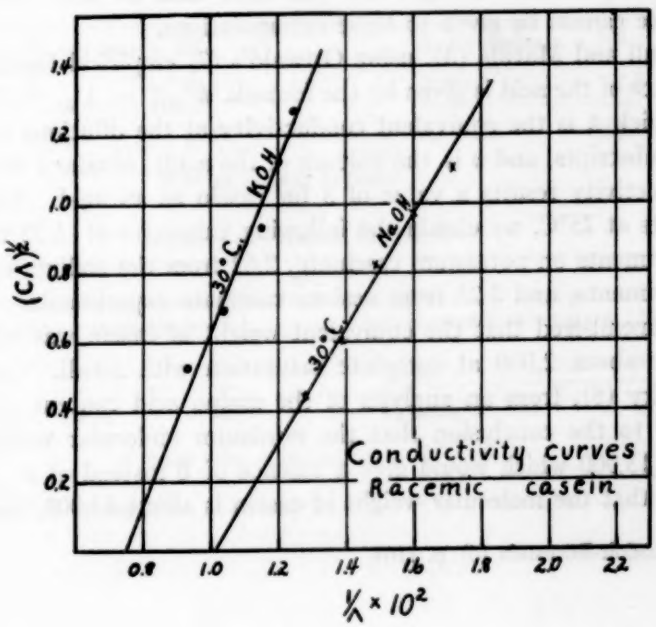
1.4
1.2
1.0
0.8
0.6
0.4
0.2
(CΛ)½
KOH
30°C.
NaOH
30°C.
Conductivity curves
Racemic casein
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
1/Λ × 10²

FIG. 4.

The extrapolation curves are given in Figs. 1 to 4.

In Table II the values of Λ_0 are given which were obtained by extrapolation, and the mobilities of the casein ion at different temperatures which were found by subtracting the mobilities of the cation from the equivalent conductivity at infinite dilution. The values of the mobilities of Na^+, K^+, and Cs^+ at different temperatures were calculated from data taken from Landolt-Börnstein *Tabellen* (6[1]), on the mobilities of ions at 18°C. and their temperature coefficients. The average values of casein ion mobilities obtained are 35 mhos for 25°C., 47 mhos for 30°C., and 65 mhos for 35°C. The value of the casein ion mobility at 30°C., agrees very well with the average result obtained from transference determinations, namely 45.3. Since all transference experiments were carried out at 30°C., the data do not permit the checking of the mobilities which were found by the conductivity method for other temperatures. Pauli and Matula, who worked at 25°C., extrapolated their results by the method already mentioned and obtained values for the mobility of the casein ion between 28 and 32.5 mhos, in solutions containing between approximately 5 to 10 cc. of 0.1 N alkali per gm. of casein. However, with the meager and uncertain data at hand, much confidence cannot be given to these extrapolations.

Pauli and Matula (3), using Ostwald's (7) empirical formula (the valence of the acid is given by the formula $\Lambda_{1024} - \Lambda_{32} = 10 \times n$, in which Λ is the equivalent conductivity at the dilutions given by the subscripts, and n is the valence of the acid), obtained from their conductivity results a value of 3 for casein as an acid. Using our results at 25°C. we obtain the following values for n: 3.35 from the experiments on potassium caseinate, 2.62 from the sodium caseinate experiments, and 3.25 from cesium caseinate experiments. It is to be remembered that the equivalent weight of casein was estimated to be about 2,000 at complete saturation with alkali. Cohn and Hendry (8), from an analysis of the amino acid content of casein, came to the conclusion that the minimum molecular weight is at least 13,000 which would give a valence of 6 instead of 3. On the basis that the molecular weight of casein is about 13,000, Cohn and

[1] Landolt-Börnstein (6), p. 1104.

TABLE II.

Mobility of the Casein Ion at Different Temperatures.

25°C.

Sodium caseinate.	Potassium caseinate.	Cesium caseinate.
A_0 Na caseinate = 83	A_0 K caseinate = 111	A_0 Cs caseinate = 104
+	+	+
A_0 Na = 51.2	A_0 K = 74.8	A_0 Cs 78.1
A_0 casein = 31.8	A_0 casein = 36.2	A_0 casein = 25.9*

Average value of A_0 casein = 35

30°C.

A_0 Na caseinate = 103	A_0 K caseinate = 132	A_0 Cs caseinate = 128
+	+	+
A_0 Na = 56.2	A_0 K = 81.4	A_0 Cs 85.3
A_0 casein = 46.8	A_0 casein = 50.6	A_0 casein = 42.7

Average value of A_0 casein = 47

35°C.

A_0 Na caseinate = 127	A_0 Cs caseinate = 156
+	+
A_0 Na = 61.5	A_0 Cs = 92.5
A_0 casein = 65.5	A_0 casein = 63.5

Average value of A_0 casein = 65

Mobility of Racemic Casein Ion.

30°C.

A_0Na racemic caseinate = 99	A_0 K racemic caseinate = 133
+	+
A_0 Na = 56.2	A_0 K = 81.4
A_0 racemic casein = 42.8	A_0 racemic casein = 51.6

Average value of A_0 racemic casein = 47

* Not used in average.

Hendry (Table IX of their paper), also calculate that there are 19 glutamic acid molecules, 4 aspartic acid molecules, 4 tyrosine molecules, and 8 hydroxyglutamic acid molecules, making a total of thirty-five potentially free acid groups. From this, on subtracting the number of these molecules, namely 12, which contain acid amide groups as shown by the ammonia content, a value of twenty-three is obtained for the number of acid groups which are probably free in the casein molecule. If we accept Cohn and Hendry's figures, we can calculate the number of acid groups neutralized in casein solu-

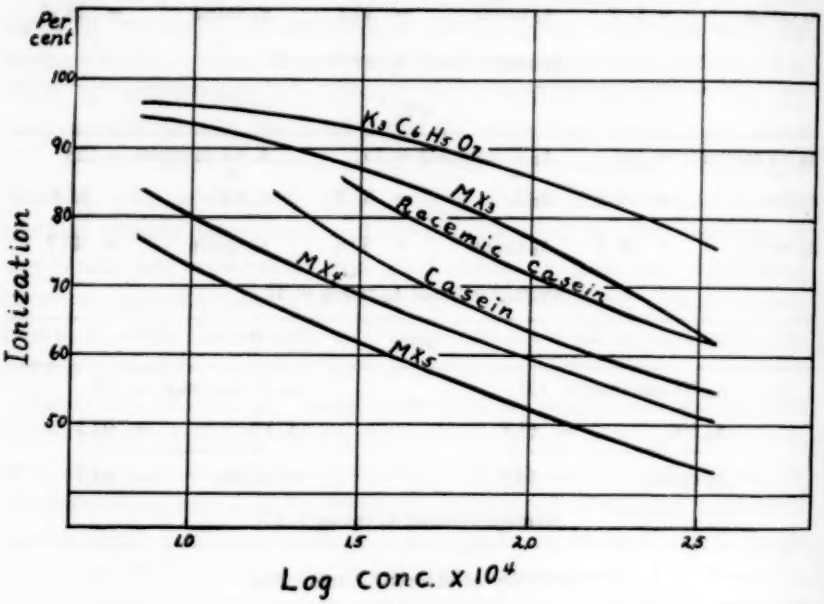

FIG. 5.

tions of about pH = 7 as follows: The maximum combining capacity of casein for bases is approximately 15.9 cc. of 0.1 N alkali per gm. Then in solutions with 5 cc. of 0.1 N alkali per gm. of casein (pH = 6.5) the number of acid groups combined is $\frac{5}{15.9} \times 23$, or seven groups.

We have still another way of approaching this problem. Randall (9) has pointed out that on plotting the degree of ionization obtained from conductivity against the concentration, or the square root of the concentration, salts of the same valence type give prac-

tically the same curves. Thus he points out that the chlorides, bromides, and iodides of lithium, sodium, potassium, rubidium, cesium, and ammonium are all ionized to about the same extent, and so on for other types of salts. By plotting the ionization curve of our casein solutions we can compare it with the curves of the salts of the various valence types and observe to which type it most closely approaches. This we have done in Fig. 5, but instead of using the concentration or the square root of the concentration, we have used

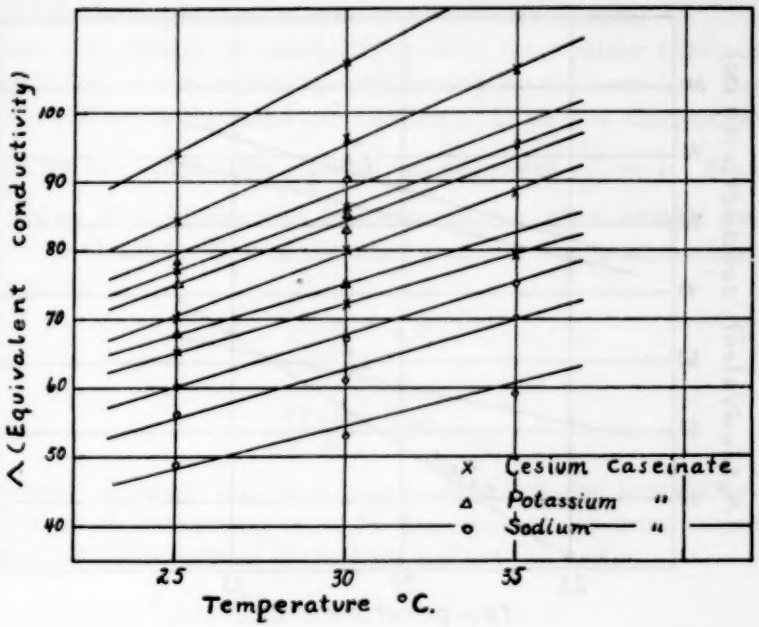

FIG. 6.

the logarithm of the concentration as one axis. The data for the comparison curves we have taken from Table II of Randall's paper. MX_4 and MX_5 are tetrasodium penta carboxylate and penta sodium benzene penta carboxylate. MX_3 represents not actual data, but is instead a theoretical curve made by taking $(1 - a) = \frac{3}{2}(1 - a)$ for Tl_2SO_4. The curves for casein and racemic casein are drawn from averaged results of the data for all of our conductivity experiments. It is to be noticed that while the casein curve is not exactly similar

to any of the comparison curves, it comes nearer to being like the MX_4 curve than any other. All this has no deep significance except that it illustrates the dangers of drawing far reaching conclusions from empirical rules made for another class of substances, as Pauli and Matula have done, when from Ostwald's rule they conclude that the neutral salts of casein with alkali have the formula "B_3 caseinate."

From the conductivity determinations made at several temperatures we can now proceed to make certain generalizations, although,

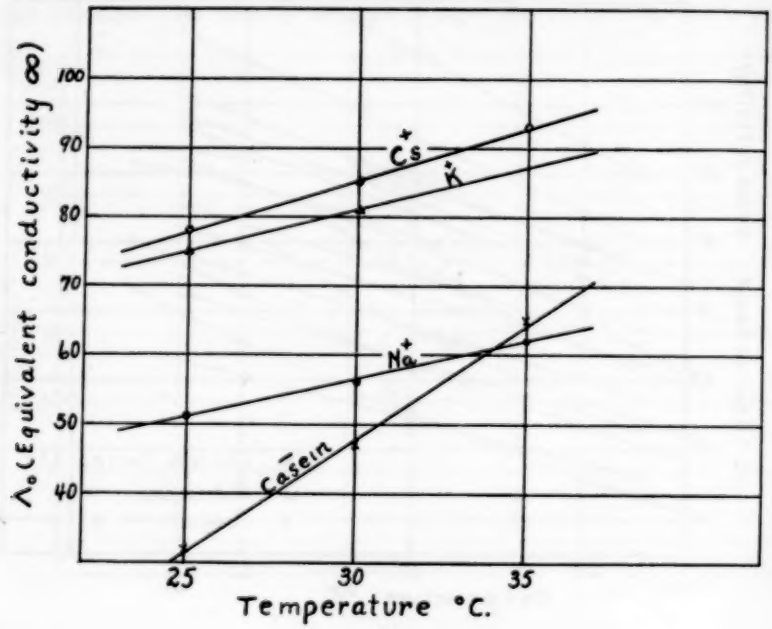

FIG. 7.

due to the meagerness of the data and the restricted range of temperatures, the generalizations made must be taken with reserve. The change of conductivity with temperature for strong electrolytes has been shown to follow a linear relationship (10). It is of interest to determine whether the conductivity of the alkali caseinate solutions also has a linear temperature relationship. That this is so over the range covered by us is shown in Fig. 6, in which the equivalent conductivity is plotted against the temperature for cesium caseinate

and for sodium caseinate. The results with the same solution measured at different temperatures are used in each case.

In Fig. 7 the temperature curves are plotted for the mobility of the casein ion, together with curves of sodium and potassium ion mobilities for comparison. The points on the casein ion curve are the average values obtained by extrapolating our conductivity data. The graph in this case too, is a straight line, which favors the view that typical salt-like compounds are formed by the combination of casein (and by implication, of other proteins) with alkalies.

Since the change of conductivity with temperature follows a straight line, we can express the relationship by the formula $\Lambda t = \Lambda n + bt$ in which Λn and b are constants. If we now differentiate we get for the temperature gradient the expression $\frac{d\Lambda}{dt} = b$. Since the change of mobilities with temperatures also gives straight line graphs, we obtain for their temperature gradients similar expressions; for example

$$\frac{d\ \Lambda_0\ \text{cation}}{dt} = b'$$

and

$$\frac{d\ \Lambda_0\ \text{anion}}{dt} = b''$$

It becomes apparent that at complete ionization the temperature gradient of the compound must be the sums of the temperature gradients of the mobilities of the ions, and we can write:

$$\frac{d\ \Lambda_0}{dt} = \frac{d\ (\Lambda_0\ \text{cation} + \Lambda_0\ \text{anion})}{dt} = b' + b''$$

From Fig. 7 we obtain for the gradient of the casein ion mobility, 3 mhos per degree centigrade; for the sodium ion 1.05 mhos, for potassium 1.32 mhos, and for cesium 1.44 mhos per degree. Then by adding the value for each cation respectively to the value for the casein ion, there are obtained as temperature gradients on complete ionization, for sodium caseinate, 4.05 mhos, for K caseinate, 4.32 mhos, and for cesium caseinate, 4.44 mhos per degree centigrade. No explanation as to why the gradient for the casein ion should be so large in comparison to the other known ions is offered at this time.

Taking into consideration what we know about the size of the protein molecule, the value for the mobility of the casein ion which was obtained by us from transport number experiments and conductivity experiments seems exceedingly high. At 30°C. we have an average value of 45.3 mhos for the casein ion mobility, which is higher than the value of the lithium ion (43.7 mhos) and about equal to that of the acetate ion (45 mhos). J. W. McBain has published a very interesting theory to account for the high conductivity of soap solutions, which he believes also applies to other colloidal electrolytes including proteins in solution. In the paper by McBain and Salmon (11) they state, "Colloidal electrolytes are salts in which an ion has been replaced by a heavily hydrated polyvalent micelle that carries an equivalent sum-total of electrical charges and conducts electricity just as well or even better than the simple ion it replaces." McBain gives the following mechanical explanation, based on Stoke's law, for the high mobility. For a sphere of radius r moving through a liquid of viscosity n, the velocity V will be $V = \frac{F}{6\pi nr}$, in which F is the force and the other terms have the meaning previously given. In conductivity experiments, the force F is due to the electrical charge on the ion and equals 1 faraday of electricity per gm. ion. If now a number of ions coalesce, say t ions, the force will become tF, and the radius of the sphere will now become $t^{\frac{1}{3}}r$. The new velocity will then be $V_1 = t^{\frac{2}{3}}\frac{F}{6\pi nr}$ which is $t^{\frac{2}{3}}$ times the old one, other factors remaining the same. This also means that there would be an increased mobility by the same amount, if t ions should coalesce to form one ion with t times the previous charge. However, McBain claims that the actual increase will be less than this calculated amount, since the enhanced electrostatic potential will attract water molecules and other material which will cut down the mobility. The aggregate that is produced will become a heavily hydrated micelle. McBain has brought forth much experimental data to support this view for soap solutions. For the proteins, while what little data we have supports it, the information is as yet too small for a sweeping conclusion. Another point that has some

bearing upon McBain's idea as applied to protein solutions is the large increase in the mobility of the casein ion with increasing temperature, as found by ourselves. On the basis of McBain's idea this can be explained by larger aggregate formation or by decreased hydration of the existing ion or micelle. That there is increased aggregation is extremely unlikely; but if we accept viscosity as a measure of hydration in protein solutions then dehydration does occur as the viscosity decreases with increased temperatures. McBain also found a high conductivity temperature coefficient in soap solutions, which he explains by assuming a dehydration of the complex soap micelle.

In the solutions with which we worked there could be no complicating effect of hydrogen or hydroxyl ions since the solutions were practically neutral, as shown by indicators and by the hydrogen electrode. That leaves only complex casein ions, as Robertson has supposed, or casein ions and inorganic cations, that take part in the carrying of the electric current.

We believe that our work can best be interpreted by the view that casein ions and inorganic cations are present in the solutions and they act as the carriers of the electric current.

SUMMARY.

1. The results of conductivity experiments with alkali caseinate solutions are given and a graphical method of extrapolation, which gives a straight line, is described. The results of the conductivity experiments are shown to be in accord with the results of the previous transference experiments.

2. The change of conductivity of the alkali caseinate solutions with temperature is shown to follow a straight line relationship.

3. The high value of the mobility which was obtained for the casein ion and the high temperature gradient are discussed in relation to McBain's theory of colloidal electrolytes.

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STUDIES ON THE FORMATION AND IONIZATION OF THE COMPOUNDS OF CASEIN WITH ALKALI.

III. The Electrochemical Behavior of Racemic Casein.*

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(Accepted for publication, October 1, 1924.)

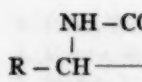
To understand Robertson's (1) prediction for the possible electrochemical behavior of racemized proteins, it will be necessary to review briefly the subject of racemized proteins and Dakin's theory of the change which takes place in the course of racemization.

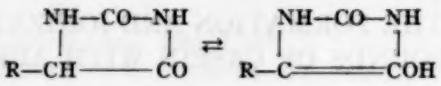
Kossel and Weiss (3), working on the protamines, observed that solutions of these proteins in approximately 0.5 N NaOH or $Ba(OH)_2$, which were left standing at body temperature, decreased in optical activity until a constant minimum was reached. They showed that this racemization is different from ordinary amino acid racemization; solutions of amino acids, namely histidine and arginine, treated in the above manner show no changes in optical activity for periods of over several months.

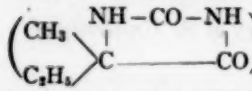
Dakin took up the study of this phenomenon, and in a paper with Dudley (4) showed that racemized casein and caseose, when fed to dogs, are totally undigested and unabsorbed. These workers further found that these racemic proteins are not attacked by trypsin, pepsin, or erepsin and that they are very resistant to bacterial action. Ten Broeck (5), Schmidt (2), and Kahn and McNeil (6) showed that racemic proteins are non-antigenic. By racemization and subsequent hydrolysis of the proteins and determination of the optical activity of the various amino acids, Dakin (7) worked out a method of differentiating between closely related proteins, *e.g.* albumins of hen and of duck eggs.

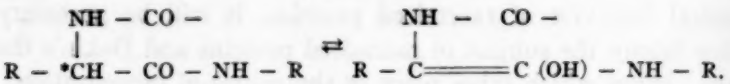
To account for the changes in optical activity, Dakin (8) proposed the following theory, to which he was led by a consideration of the racemization of the hydantoins. He had found that the hydantoins, which have the formula

* Aided by a grant from the Research Board of the University of California.

 , lose their optical activity in alkaline solutions. Dakin accounted for this by assuming that the hydantoins exist in the following equilibrium.

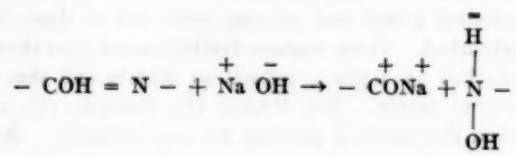


Since in going from right to left in this equation equal quantities of *d* and *l* forms would be formed, the compound would become racemized. As a clinching argument in favor of this mechanism, Dakin showed that methylethylhydantoin  which has no labile hydrogen, does not undergo racemization. As the peptide linkage in the protein is very closely related to the hydantoin structure, Dakin (9) proposed that the racemization of proteins takes place in an analogous manner, namely according to the equation:

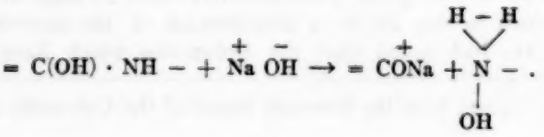


In this way it is seen that the asymmetric carbon marked * loses its asymmetry with the formation of the double bond. That the optical activity is not completely lost is explained by the terminal amino acids not being racemized, since their carboxyl groups are not bound in a peptide linkage.

Robertson (1) pointed out that if Dakin's theory is correct it would be necessary that racemic proteins dissociate in a manner different from that proposed by his (Robertson's) theory. According to this theory the alkali salt of a normal protein would give the ions



while for a racemic protein it would be necessary to assume that its alkali salts would ionize to give the ions



In the first case we have doubly charged ions produced and in the last singly charged ions. This hypothesis can be tested in the following way. If the ions produced by racemic casein carry only half of the charge of the ions of normal casein, then (1) it should take only half of the quantity of electricity which is required to deposit a given quantity of casein to deposit an equal quantity of racemic casein. In other words, the electrochemical equivalent of racemic casein should be twice that of ordinary casein. And (2) since in the two forms everything should be equal but the charge on the ions, the electrical conductivity of ordinary casein solutions should be double that of similar racemic casein solutions. This offers a good opportunity of checking Robertson's and Dakin's hypotheses against each other. If the results obtained agree with what would be expected from theoretical considerations, they should give strong evidence in favor of both theories. However, if the findings were negative we would be just where we were before, since the experiments would not tell us which of the two hypotheses is incorrect. We carried out experiments to test this idea, with the results shown in Tables I and II.

We chose casein for this work because Robertson had worked mainly with this protein. Racemic casein was prepared according to Dakin's directions (4). The process was followed with the aid of the polariscope until the rotation remained constant. In a 2 dm. tube, using white light, the reading of a 10 per cent casein solution in half normal NaOH fell from $-10.0°$ to $-1.5°$ in 2 weeks. The solution was then neutralized to Congo red with acetic acid, and the racemic casein which precipitated was collected and washed with distilled water. It was then broken up into small particles, placed in tall glass bottles, and washed with distilled water by decantation several times a day for several weeks. It was then dried over H_2SO_4 at 40°C., and ground up into a powder.

Table I gives a comparison of the electrochemical equivalents which were found in solutions of casein and of racemic casein in dilute alkali. The details of the methods which were used to obtain the values given in Tables I and II were described in previous papers of this series. In the columns marked Q, the amounts of casein or of racemic casein are given deposited on the anode by the

passage of 1 millifaraday of electricity through the solution. Under K, are given the products obtained by multiplying the values Q by B, the number of cc. of 0.1 N alkali used to dissolve 1 gm. of protein. This gives practically a constant value. For the present purpose it is to be observed that this constant value is practically the same for both casein and racemic casein. This is not in accord with the prediction to be made from the Robertson theory. Similarly, an ex-

TABLE I.

Comparison of Electrochemical Equivalents of Casein and Racemic Casein (Average Values).

KOH

Casein.				Racemic casein.			
	B	*Q*	*K*		*B*	*Q*	*K*
per cent				*per cent*			
2.90	5.00	1.68	8.40	2.04	5.40	1.66	8.95
2.57	6.42	1.37	8.80	2.40	6.47	1.32	8.55
2.42	8.30	1.08	8.95	1.60	8.65	0.98	8.50

NaOH

Casein.				Racemic casein.			
	B	*Q*	*K*		*B*	*Q*	*K*
per cent				*per cent*			
2.85	5.60	1.78	9.98	1.95	5.40	1.64	8.85
2.52	7.00	1.34	9.40	1.96	7.47	1.16	8.65
1.70	8.25	1.07	8.80	1.90	7.65	1.18	9.04

B = cc. 0.1 N alkali per gm.
Q = electrochemical equivalent per millifaraday.
K = constant.

amination of Table II shows a very close agreement in the conductivity values for similar solutions of casein and racemic casein, which again is not in accord with Robertson's idea.

However, this cannot be taken as a very conclusive proof against Robertson's theory for the reason already stated, that negative results do not tell which of the theories (Robertson's or Dakin's) is correct.

Another reason for not taking the results of the electrochemical equivalent and conductivity determinations as conclusive evidence against Robertson's theory is that we do not know but that racemic casein has undergone some other chemical change besides racemization which would affect its electrochemical properties. That extensive chemical changes take place in the casein solution during the course of racemization is easily demonstrated by the fact that the

TABLE II.

Comparison of Conductivities of Casein and Racemic Casein Solutions.

Approximately 5 cc. 0.1 N NaOH per gm. (30°).

Alkali concentration in equivalents.	Casein. $k \times 10^4$	Racemic casein. $k \times 10^4$	Casein. A	Racemic casein. A
0.02	10.55	12.05	53.0	60.1
0.010	6.10	6.70	61.0	67.0
0.005	3.35	3.60	67.0	72.0
0.0025	1.85	1.95	73.0	78.0
0.00125	1.05	1.07	84.0	85.2

Casein 5 cc. 0.1 N KOH per gm. (30°).

Alkali concentration in equivalents.	Casein. $k \times 10^4$	Racemic casein. $k \times 10^4$	Casein. A	Racemic casein. A
0.02	14.90	16.10	75.5	80.5
0.015	12.5		83.5	
0.010	8.50	8.70	85.0	87.0
0.005	4.50	4.80	90.0	96.0
0.0025	2.45	2.70	98.0	101.0
0.00125	1.35	1.40	108.0	112.0

K = specific conductivity.

racemic casein which was obtained constituted only about one-fifth of the original casein in amount, and also by there being a considerable evolution of ammonia during the process.

We endeavored to obtain some information as to whether racemic casein has undergone extensive changes by determining the nitrogen distribution according to the Van Slyke method. A determination of the total nitrogen per cent by the Kjeldahl method gave 13.5 per cent for one sample, 13.16 per cent for another sample, against 14.6

per cent for the original casein used. This change in nitrogen content may be due to either of two possibilities: (1) racemic casein is a complex split product of the casein, or (2) the nitrogen is lost by hydrolysis of part or all of the amide nitrogen. It has been shown by Osborne and Nolan (10) that the splitting off of this amide nitrogen takes place very readily in alkaline solutions. The results of the nitrogen distribution analysis are given in Table III. Comparison is made with the figures which have been published for casein by Van Slyke (11).

TABLE III.

Van Slyke Nitrogen Distribution Analysis.

Casein (11).		Racemic casein I.		Racemic casein II.	
Total N.	14.6 per cent.	13.5 per cent.	Corrected to 14.6 per cent.	13.16 per cent.	Corrected to 14.6 per cent.
Amide N	10.27	4.75	4.4	3.13	2.8
Humin N	1.28	1.25		1.18	
Total basic N	23.9	22.5	20.4	24.8	22.4
Arginine N	7.4	8.25	7.6	8.57	7.75
Histidine N	6.2	2.25		3.90	
Lysine N	10.3	12.00	11.1	12.05	10.08
Non-basic N	62.9	68.11	63.0	69.5	63.0
Monoamino N	55.8	55.9	51.8	60.5	54.5
Non-amino N	7.13	12.2	11.3	9.0	8.15
Total	98.5	96.6		98.65	

In the columns marked "Corrected to 14.6 per cent total N" there is given the composition on the basis of a 14.6 per cent total nitrogen content, for the purpose of a better comparison with the Van Slyke figure. On first sight it appears that there is a considerable change in composition, especially for histidine, lysine, and the non-amino nitrogen. The amount of amide nitrogen is also very much lower than in ordinary casein. However, if we take the data for total basic nitrogen and for total non-basic nitrogen we find the agreement between casein and racemic casein to be quite close. Also arginine, which is susceptible to the most accurate analysis according to the Van Slyke scheme, gives very good agree-

ment with Van Slyke's figures. From this data it would be very difficult to decide as to whether there had been any change in the amino acid content on racemization.

From the amide nitrogen figures, we can calculate how much of the difference in nitrogen content between casein and racemic casein is due to hydrolysis of amide groups. Carrying out this calculation, we get 0.86 per cent for Sample I as the loss due to loss in amide nitrogen, which is 78 per cent of the difference in nitrogen content; and for Sample II we obtain 1.1 per cent, which is 76.5 per cent of

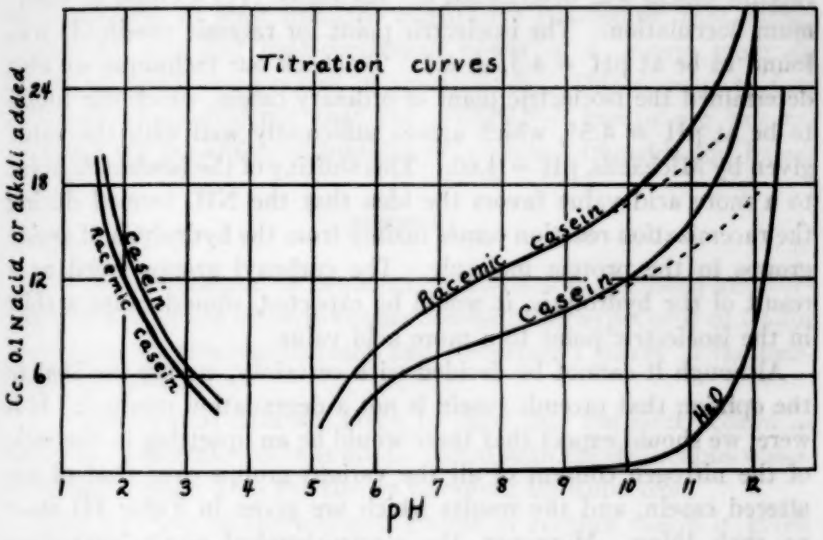


FIG. 1. Dotted lines indicate correction for water blank.

the difference in nitrogen content. Since the method of determining amide nitrogen gives good results the loss can hardly be put down as due to errors in the determination. It seems quite evident that some other nitrogen group in the molecule is hydrolyzed beside the amide group. As confirming evidence, we found the maximum combination of alkali by racemic casein to be more than that of unaltered casein by an amount corresponding to the difference in amide nitrogen (*cf.* Fig. 1). The difference in amide nitrogen between ordinary casein and the sample of racemic casein (1) is 0.86 per cent of the protein, *i.e.* each gm. of racemic casein (1) contains 0.0086 gm. of

ammonia nitrogen less than each gm. of ordinary casein. If this results from the hydrolysis of the acid amide groups, then 1 gm. of racemic casein should be able to combine with 6.15 cc. of 0.1 N alkali more than is required for 1 gm. of ordinary casein. Fig. 1 shows that the maximum combining capacity of a gm. of racemic casein is 6 cc. of 0.1 N alkali greater than for normal casein.

It was thought that it would be of interest to know how the isoelectric point was affected by changes in protein structure, in this case by the hydrolysis of amide groups, so the isoelectric point of racemic casein was determined by Michaelis' (12) method of maximum flocculation. The isoelectric point for racemic casein (I) was found to be at pH = 4.3, ± 0.1. To check our technique we also determined the isoelectric point of ordinary casein, which was found to be at pH = 4.55, which agrees sufficiently well with the value given by Michaelis, pH = 4.60. This shifting of the isoelectric point to a more acid value favors the idea that the NH_3 formed during the racemization reaction comes mainly from the hydrolysis of amide groups in the protein molecule. The carboxyl groups freed as a result of the hydrolysis, it would be expected, should cause a shift in the isoelectric point to a more acid value.

Although it cannot be decided with certainty, we are inclined to the opinion that racemic casein is not a degradation product. If it were, we should expect that there would be an upsetting in the ratio of the nitrogen content of all the various groups over that of unaltered casein, and the results which are given in Table III show no such thing. Moreover, the electrochemical equivalents given in Table I show that in similar solutions the mass associated with a given charge is very nearly the same for casein and for racemic casein, showing that the molecule of racemic casein must be very nearly as large as that of unaltered casein.

DISCUSSION.

It has been pointed out by us elsewhere (13)[1] that the combining power of gelatin for HCl is equal to the sum of the ε-amino group of

[1] The work of Cohn and Berggren (Cohn, E. J., and Berggren, R. E. L., *J. Gen. Physiol.*, 1924–25, vii, 45) is essentially in harmony with views previously expressed by us. They were unaware of our publication.

its lysine content plus the amino group contained in the guanidine group of its content of arginine. Good agreement has also been obtained between the combining powers of casein, gelatin, and gliadin for alkali, and their respective contents of the dibasic acids, glutamic, aspartic, hydroxyglutamic, and tyrosine, less the amount of these acids which appear to be combined as acid amides. While it is frankly admitted that our knowledge concerning the ability of the various amino acids especially proline,[2] in the protein molecule, to combine with acids and alkali is still incomplete, nevertheless our data indicate, at least from the qualitative standpoint, that within limits the acid and base-combining power of a protein molecule lies in its particular content of amino acids. This appears to furnish additional evidence that the $-COHN-$ groups are not responsible for the ability of proteins to combine with acids and bases.

SUMMARY.

1. The phenomenon of protein racemization is discussed and certain deductions are made in connection with the hypothesis of Dakin to account for this phenomenon and Robertson's theory of the ionization of proteins.

2. Experimental data are given to show that the electrochemical behavior of racemic casein is not in accord with the deductions which have been drawn from the theory advanced by Robertson.

3. An analysis of the nitrogen groups of racemic casein is given and compared with a similar analysis of normal casein. From these analyses and from the electrochemical equivalent of racemic casein, it is concluded that except for the hydrolysis of amide groups, racemic casein is probably not a degradation product of casein.

4. Considerable evidence is presented against the view that the $-COHN-$ groups take part in the reactions of the protein molecule with acids and with bases.

[2] Experimental work to determine the dissociation constants of proline is under way.

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